- Failing or nearby septic tank systems
- Exfiltration from sanitary sewers in poor repair
- Leaking underground storage tanks and pipes
- Landfill seepage
- Hazardous waste disposal sites
- Naturally occurring toxicants and pollutants due to surrounding geological or natural environment

Leaks from underground storage tanks and pipes are a common source of soil and groundwater pollution and may lead to continuously contaminated dry-weather entries. These situations are usually found in commercial operations, such as gasoline service stations, or industries involving the piped transfer of process liquids over long distances and the storage of large quantities of fuel, e.g., petroleum refineries. Pipes that are plugged or collapsed, as well as leaking storage tanks, may cause pollution when they release contaminants underground which can infiltrate through the soil into stormwater pipes.

The most common potential nonstormwater entries, which have been identified by a review of documented case studies for commercial and residential areas by Lalor (1993) and Pitt et al. (1993) included:

- Sanitary wastewater sources:
  - Raw sanitary wastewater from improper sewerage connections, exfiltration, or leakage
  - Effluent from improperly operating, designed, or nearby septic tanks
- Automobile maintenance and operation sources:
  - Car wash wastewaters
  - Radiator flushing wastewater
  - Engine degreasing wastes
  - Improper oil disposal
  - Leaky underground storage tanks
- Relatively clean sources:
  - Lawn runoff from over-watering
  - Direct spraying of impervious surfaces
  - Infiltrating groundwater
  - Water routed from preexisting springs or streams
  - Infiltrating potable water from leaking water mains
- Other sources:
  - Laundry wastewaters
  - Noncontact cooling water
  - Metal plating baths
  - Dewatering of construction sites
  - Washing of concrete ready-mix trucks
  - Sump pump discharges
  - Improper disposal of household toxic substances
  - Spills from roadway and other accidents

From the above list, sanitary wastewater is the most significant source of bacteria, while automobile maintenance and plating baths are the most significant sources of toxicants. Waste discharges associated with the improper disposal of oil and household toxicants tend to be intermittent and low volume. These wastes may therefore not reach the stormwater outfalls unless carried by higher flows from another source, or by stormwater during rains.

Human Health Problems Caused by Inappropriate Discharges

There are several mechanisms through which exposure to stormwater can cause potential human health problems. These include exposure to stormwater contaminants at swimming areas affected by stormwater discharges, drinking water supplies contaminated by stormwater discharges, and the consumption of fish and shellfish that have been contaminated by stormwater pollutants. In receiving waters having only stormwater discharges, it is well known that inappropriate sanitary and other wastewaters are also discharging through the storm drainage system. The most serious problems appear to be associated with the presence of potential pathogens in problematic numbers. Contact recreation in pathogen-contaminated waters has been studied at many locations. The sources of the pathogens are typically assumed to be sanitary sewage effluent or periodic industrial discharges from certain food preparation industries (especially meat packing and fish and shellfish processing). However, several studies have investigated pathogen problems associated with stormwater discharges. It has generally been assumed that the source of the pathogens in the stormwater is inappropriate sanitary connections. However, stormwater unaffected by these inappropriate sources still contains high counts of pathogens that are also found in surface runoff samples from many urban surfaces. Needless to say, sewage contamination of urban streams is an important issue that needs attention during an urban water assessment investigation. Obviously, inappropriate discharges must be identified and corrected as part of any effort to clean up urban streams. If these sources are assumed to be nonexistent in an area and are therefore not considered in the stormwater management activities, incorrect and inefficient management decisions are likely, with disappointing improvements in the receiving waters.

A number of issues emerged from the individual projects of the U.S. EPA's NURP (EPA 1983a). One of these issues involved illicit connections to storm drainage systems and was summarized as follows in the Final Report of the NURP executive summary: "A number of the NURP projects identified what appeared to be illicit connections of sanitary discharges to stormwater sewer systems, resulting in high bacterial counts and dangers to public health. The costs and complications of locating and eliminating such connections may pose a substantial problem in urban areas, but the opportunities for dramatic improvement in the quality of urban stormwater discharges certainly exist where this can be accomplished. Although not emphasized in the NURP effort, other than to assure that the selected monitoring sites were free from sanitary sewage contamination, this BMP (best management practice) is clearly a desirable one to pursue." The illicit discharges noted during NURP were especially surprising, because the monitored watersheds were carefully selected to minimize factors other than stormwater. Presumably, illicit discharge problems in typical watersheds would be much worse. Illicit entries into urban storm sewerage were identified by flow from storm sewer outfalls following substantial dry periods. Such flow could be the result of direct "illicit connections" as mentioned in the NURP final report, or could result from indirect connections (such as contributions from leaky sanitary sewerage through infiltration to the separate storm drainage). Many of these dry-weather flows are continuous and would therefore also occur during rain-induced runoff periods. Pollutant contributions from the dry-weather flows in some storm drains have been shown to be high enough to significantly degrade water quality because of their substantial contributions to the annual mass pollutant loadings to receiving waters.

In many cases, sanitary sewage was an important component (although not necessarily the only component) of the dry-weather discharges from storm drainage systems that have been investigated. From a human health perspective (associated with pathogens), it may not require much raw or poorly treated sewage to cause a receiving water problem. However, at low discharge rates, the DO receiving water levels may be minimally affected. The effects these discharges have on the receiving waters is therefore highly dependent on many site-specific factors, including frequency and quantity of sewage discharges and the creek flows. In many urban areas, the receiving waters are small creeks in completely developed watersheds. These creeks are the most at risk from these discharges as dry baseflows may be predominantly dry-weather flows from the drainage systems. In Tokyo (Fujita 1998), for example, numerous instances were found where correcting inappropriate sanitary sewage discharges resulted in the urban streams losing all of their flow. In cities adjacent to large receiving waters, these discharges likely have little impact (such as DO impacts from Nashville CSO discharges on the Cumberland River; Cardozo et al. 1994). The presence of pathogens from raw or poorly treated sewage in urban streams, however, obviously presents a potentially serious public health threat. Even if the receiving waters are not designated as water contact recreation, children often play in small city streams.

Assessment Strategies for Identifying Inappropriate Discharges to Storm Drainage

The following is a summary of the strategy developed by Lalor (1993) and Pitt et al. (1993) for the EPA to support the outfall screening activities required by the National Pollutant Discharge Elimination System (NPDES) Stormwater Permit Program to identify and correct inappropriate discharges to storm drainage systems. Those documents should be consulted for more detailed information. The methods summarized here require the use of multiple indicators used in combination. The evaluation procedures outlined range from the most basic, requiring minimal information, to more complex, requiring additional analyses.

The detection and identification of flow components require the quantification of specific characteristics of the observed combined flow. Lalor (1993) developed a simple test suite that tested very reliably in field verification trials. This method requires the analysis of detergents, fluoride, ammonia, and potassium, plus noting obvious indicators. The characteristics of most interest should be relatively unique for each potential flow source. This will enable the presence of each flow source to be indicated, based on the presence (or absence) of these unique characteristics. The selected characteristics are termed *tracers*, because they have been selected to enable the identification of the sources of these waters. These methods can be used in many areas, although the selection of the specific tracers might vary if the likely source flows are different. This section also discusses other methods used to indicate sources of contaminants, such as fingerprinting hydrocarbon residuals and newly available analytical methods that are very specific to individual sources.

Investigations designed to determine the contribution of urban stormwater runoff to receiving water quality problems have led to a continuing interest in inappropriate connections to storm drainage systems. Urban stormwater runoff is traditionally defined as that portion of precipitation which drains from city surfaces and flows via natural or man-made drainage systems into receiving waters. In fact, urban stormwater runoff also includes waters from many other sources which find their way into storm drainage systems. Sources of some of this water can be identified and accounted for by examining current NPDES permit records for permitted industrial wastewaters that can be legally discharged to the storm drainage system. However, most of the water comes from other sources, including illicit and/or inappropriate entries to the storm drainage system. These entries can account for a significant amount of the pollutants discharged from storm sewerage systems (Pitt and McLean 1986).

In response to the early studies that indicated the importance of stormwater discharge effects on receiving waters, provisions of the Clean Water Act (1987) now require NPDES permits for stormwater discharges. Permits for municipal separate storm sewers include a requirement to effectively prohibit problematic nonstormwater discharges, thereby placing emphasis on the elimination of inappropriate connections to urban storm drains. Section 122.26 (d)(1)(iv)(D) of the rule specifically requires an initial screening program to provide means for detecting high levels of pollutants in dry-weather flows, which should serve as indicators of illicit connections to the storm sewers. To facilitate the application of this rule, the EPA's Office of Research and Development's Storm and Combined Sewer Pollution Control Program and the Environmental Engineering & Technology Demonstration Branch, along with the Office of Water's Nonpoint Source Branch, supported research for the investigation of inappropriate entries to storm drainage systems (Pitt et al. 1993). This research was designed to provide information and guidance to local agencies by (1) identifying and describing the most common potential sources of nonstormwater pollutant entries into storm drainage systems; and (2) developing an investigative methodology that would allow a user to determine whether significant nonstormwater entries are present in a storm drain, and then to identify the type of source, as an aid to determining the location of the source. An important premise for the development of this methodology was that the initial field screening effort would require minimal effort and expense, but would have little chance of missing a seriously contaminated outfall. This screening program would then be followed by a more in-depth analysis to more accurately determine the significance and source of the nonstormwater pollutant discharges.

The approach presented in this research was based on the identification and quantification of clean baseflow and the contaminated components during dry weather. If the relative amounts of potential components are known, then the importance of the dry-weather discharge can be determined. As an example, if a baseflow is mostly uncontaminated groundwater, but contains 5% raw sanitary wastewater, it is likely an important source of pathogenic bacteria. Typical raw sanitary wastewater parameters (such as BOD_5 or suspended solids) would be in low concentrations and the sanitary wastewater source would be difficult to detect. Fecal coliform bacteria measurements would not help much because they originate from many possible sources, in addition to sanitary wastewater. Expensive unique microorganism or biochemical measurements would probably be needed to detect the presence of the wastewater directly. However, a tracer may be identified that can be used to identify relatively low concentrations of important source flows in storm drain dry-weather baseflows.

The ideal tracer should have the following characteristics:

- Significant difference in concentrations between possible pollutant sources
- Small variations in concentrations within each likely pollutant source category
- A conservative behavior (i.e., no significant concentration change due to physical, chemical, or biological processes)
- Ease of measurement with adequate detection limits, good sensitivity, and repeatability

In order to identify tracers meeting the above criteria, literature characterizing potential inappropriate entries into storm drainage systems was examined. Several case studies which identified procedures used by individual municipalities or regional agencies were also examined. Though most of the investigations resorted to expensive and time-consuming smoke or dye testing to locate individual illicit pollutant entries, a few provided information regarding test parameters or tracers. These screening tests were proven useful in identifying drainage systems with problems before the smoke and dye tests were used. The case studies also revealed the types of illicit pollutant entries most commonly found in storm drainage systems.

Selection of Parameters for Identifying Inappropriate Discharge Sources

Table 6.33 is an assessment of the usefulness of candidate field survey parameters in identifying different potential nonstormwater flow sources. Natural and domestic waters should be uncontaminated (except in the presence of contaminated groundwaters entering the drainage system, for example). Sanitary sewage, septage, and industrial waters can produce toxic or pathogenic conditions. The other source flows (wash and rinse waters and irrigation return flows) may cause nuisance conditions or degrade the ecosystem. The parameters marked with a plus sign can probably be used to identify the specific source flows by their presence. Negative signs indicate that the potential source flow probably does not contain the listed parameter in adverse or obvious amounts, and may help confirm the presence of the source by its absence. Parameters with both positive and negative signs for a specific source category would probably not be very helpful due to expected wide variations.

Fecal Coliform Bacteria as Indicators of Inappropriate Discharges of Sanitary Sewage

Several investigations have relied on fecal coliform measurements as indicators of sanitary sewage contamination of stormwater. However, the use of fecal coliforms has been shown to be

Table 6.33 Candidate Field Survey Parameters and Associated Non-Stormwater Flow Sources

Parameter	Natural Water	Potable Water	Sanitary Sewage	Septage Water	Indus. Water	Wash Water	Rinse Water	Irrig. Water
Fluoride	–	+	+	+	+/–	+	+	+
Hardness change	–	+/–	+	+	+/–	+	+	–
Surfactants	–	–	+	–	–	+	+	–
Fluorescence	–	–	+	+	–	+	+	–
Potassium	–	–	+	+	–	–	–	–
Ammonia	–	–	+	+	–	–	–	+/–
Odor	–	–	+	+	+	+/–	–	–
Color	–	–	–	–	+	–	–	–
Clarity	–	–	+	+	+	+	+/–	–
Floatables	–	–	+	–	+	+/–	+/–	–
Deposits and stains	–	–	+	–	+	+/–	+/–	–
Vegetation change	–	–	+	+	+	+/–	–	+
Structural damage	–	–	–	–	+	–	–	–
Conductivity	–	–	+	+	+	+/–	+	+
Temperature change	–	–	+/–	–	+	+/–	+/–	–
pH	–	–	–	–	+	–	–	–

Note: – implies relatively low concentration; + implies relatively high concentration; +/– implies variable conditions.

From Pitt et al. 1993.

an inadequate indicator of sewage except in gross contamination situations (see also Chapter 3). Low fecal coliform levels may also cause false negative findings, as was indicated during the Inner Grays Harbor study where a storm drain outfall with a confirmed domestic sewage connection was not found to have elevated fecal coliform levels (Pelletier and Determan 1988). High fecal coliform bacteria populations were observed at storm sewer outfalls at all times in both industrial and residential/commercial areas during a study in Toronto (Pitt and McLean 1986). During the warm-weather storm sampling period, surface sheetflows were shown to be responsible for most of the observations of bacteria at the outfalls. However, during cold weather, very few detectable surface snowmelt sheetflow or snow pack fecal coliform observations were obtained, while the outfall observations were still quite high. High fecal coliform bacteria populations were also observed during dry-weather flow conditions at the storm sewer outfalls during both warm and cold weather. Leaking, or cross-connected, sanitary sewerage was therefore suspected at both study areas. Contaminated sump-pump water (from poorly operating septic tank systems in medium-density residential areas) in the Milwaukee area has been noted as a potentially significant source of bacteria to storm drainage systems (R. Bannerman, WI DNR, personal communication).

The presence of bacteria in stormwater runoff, dry-weather flows, and in urban receiving waters has caused much concern, as described in Chapter 3. However, there are many potential sources of fecal coliforms in urban areas, besides sanitary sewage. Research projects conducted in Toronto, Ontario (Pitt and McLean 1986), and in Madison, WI (R. Bannerman, WI DNR, personal communication) have investigated the abundance of common indicator bacteria, potential pathogenic bacteria, and bacterial types that may indicate the source of bacterial contamination. The monitoring efforts included sampling from residential, industrial, and commercial areas. As in many previous studies, fecal coliforms were commonly found to exceed water quality standards by large amounts during the Toronto investigations. Fecal coliform populations were very large at all land uses investigated during warm weather (typical median outfall values were 10,000 to 30,000 organisms per 100 mL). Dry-weather baseflow fecal coliform populations were found to be statistically similar to the stormwater runoff populations. The cold-weather fecal coliform populations were much lower (300 to 10,000 per 100 mL), but still exceeded the water quality standards.

Samples were obtained from many potential sources, in addition to the outfall, during the Toronto study (Pitt and McLean 1986). Source area fecal coliform populations were very similar for different land uses for the same types of areas, but different source areas within the watersheds varied significantly. Generally, roof runoff had the lowest fecal coliform populations, while roads and roadside ditches had the largest populations.

The types and concentrations of different bacteria biotypes vary for different animal sources. Quresh and Dutka (1979) found that pathogenic bacteria biotypes are present in urban runoff and are probably from several different sources. The sources (nonhuman vs. human) of bacteria in urban runoff are difficult to determine. Geldreich and Kenner (1969) caution against using the ratio of fecal coliform to fecal streptococci as an indicator, unless the waste stream is known to be "fresh." Unfortunately, urban runoff bacteria may have been exposed to the environment for some time before rain washed it into the runoff waters. Delays may also be associated with some dry-weather bacteria sources. This aging process can modify the fecal coliform to fecal streptococci ratio to make the bacteria appear to be of human origin. In fact, samples collected in runoff source areas usually have the lowest FC/FS ratio in a catchment, followed by urban runoff, and finally the receiving water (Pitt 1983). This transition probably indicates an aging process and not a change in bacteria source.

Debbie Sargeant of the Washington State Department of Ecology has prepared a summary of different methods for fecal contamination source identification. Her report is available at www.ecy.wa.gov/biblio/99345.html. She concluded that there is no easy, low-cost method for differentiating between human and nonhuman sources of bacterial contamination. Genetic fingerprinting and newly emerging PCR methods, plus combinations of indicators, are some of the recommendations made in this report to further investigate bacterial sources.

Therefore, bacteria are usually poor indicators of the presence of sanitary sewage contamination. Past use of fecal strep to fecal coliform ratios to indicate human vs. nonhuman bacteria sources in mixed and old wastewaters (such as most nonpoint waters) has not been successful and should be used with extreme caution. There may be some value in investigating specific bacteria types, such as fecal strep biotypes, but much care should be taken in the analysis and interpretation of the results. A more likely indicator of human wastes may be the use of certain molecular markers, specifically the linear alkylbenzenes and fecal sterols, such as coprostanol and epicoprostanol (Eaganhouse et al. 1988), although these may also be discharged by other carnivores (especially dogs) in a drainage ditch. Recent discussions of specific tracers for indicating sanitary sewage contamination is presented later in this discussion. The following discussion presents a more generally useful approach for identifying inappropriate discharges to storm drainage, relying on easily evaluated chemical tracers and visual observations.

Tracer Characteristics of Local Source Flows

Table 6.34 is a summary of tracer parameter measurements for Birmingham, AL by Pitt et al. (1993). This table is a summary of the "library" that describes the tracer conditions for each potential source category. The important information shown on this table includes the median and coefficient of variation (COV) values for each tracer parameter for each source category. The COV is the ratio of the standard deviation to the mean. A low COV value indicates a much smaller spread of data compared to a data set having a large COV value. It is apparent that some of the generalized relationships shown in Table 6.33 did not exist during the demonstration project. This emphasizes the need for obtaining local data describing likely source flows.

The fluorescence values shown in Table 6.34 are direct measurements from a fluorometer having general-purpose filters and lamps and at the least sensitive setting (number 1 aperture). The toxicity screening test results are expressed as the toxicity response noted after 25 minutes of exposure using an Azur Environmental Microtox unit which measures toxicity using the light output from phosfluorescent algae. The I_{25} values are the percentage light output decreases observed after 25 minutes of exposure to the sample, compared to a reference. Fresh potable water has a relatively high toxicity response because of the chlorine levels present. Dechlorinated, potable water has much smaller toxicity responses.

Table 6.34 Tracer Concentrations Found in Birmingham, AL, Waters (Mean, Standard Deviation, and Coefficient of Variation, COV)

	Spring Water	Treated Potable Water	Laundry Wastewater	Sanitary Wastewater	Septic Tank Effluent	Car Wash Water	Radiator Flush Water
Fluorescence	6.8	4.6	1020	250	430	1200	22,000
(% scale)	2.9	0.35	125	50	100	130	950
	0.43	0.08	0.12	0.20	0.23	0.11	0.04
Potassium (mg/L)	0.73	1.6	3.5	6.0	20	43	2800
	0.070	0.059	0.38	1.4	9.5	16	375
	0.10	0.04	0.11	0.23	0.47	0.37	0.13
Ammonia (mg/L)	0.009	0.028	0.82	10	90	0.24	0.03
	0.016	0.006	0.12	3.3	40	0.066	0.01
	1.7	0.23	0.14	0.34	0.44	0.28	0.3
Ammonia/potassium	0.011	0.018	0.24	1.7	5.2	0.006	0.011
(ratio)	0.022	0.006	0.050	0.52	3.7	0.005	0.011
	2.0	0.35	0.21	0.31	0.71	0.86	1.0
Fluoride (mg/L)	0.031	0.97	33	0.77	0.99	12	150
	0.027	0.014	13	0.17	0.33	2.4	24
	0.87	0.02	0.38	0.23	0.33	0.20	0.16
Toxicity	<5	47	99.9	43	99.9	99.9	99.9
(% light decrease	n/a	20	<1	26	<1	<1	<1
after 25 min, I_{25})	n/a	0.44	n/a	0.59	n/a	n/a	n/a
Surfactants	<0.5	<0.5	27	1.5	3.1	49	15
(mg/L as MBAS)	n/a	n/a	6.7	1.2	4.8	5.1	1.6
	n/a	n/a	0.25	0.82	1.5	0.11	0.11
Hardness (mg/L)	240	49	14	140	235	160	50
	7.8	1.4	8.0	15	150	9.2	1.5
	0.03	0.03	0.57	0.11	0.64	0.06	0.03
pH (pH units)	7.0	6.9	9.1	7.1	6.8	6.7	7.0
	0.05	0.29	0.35	0.13	0.34	0.22	0.39
	0.01	0.04	0.04	0.02	0.05	0.03	0.06
Color (color units)	<1	<1	47	38	59	220	3000
	n/a	n/a	12	21	25	78	44
	n/a	n/a	0.27	0.55	0.41	0.35	0.02
Chlorine (mg/L)	0.003	0.88	0.40	0.014	0.013	0.070	0.03
	0.005	0.60	0.10	0.020	0.013	0.080	0.016
	1.6	0.68	0.26	1.4	1.0	1.1	0.52
Specific conductivity	300	110	560	420	430	485	3300
(μS/cm)	12	1.1	120	55	311	29	700
	0.04	0.01	0.21	0.13	0.72	0.06	0.22
Number of samples	10	10	10	36	9	10	10

From Pitt et al. 1993.

Appropriate tracers are characterized by having significantly different concentrations in flow categories that need to be distinguished. In addition, effective tracers also need low COV values within each flow category. Table 6.33 shows the expected changes in concentrations per category, and Table 6.34 indicates how these expectations compared with the results of an extensive local sampling effort. The study indicated that the COV values were quite low for each category, with the exception of chlorine, which had much greater COV values. Chlorine is therefore not recommended as a quantitative tracer to estimate the flow components. Similar data should be collected in each community where these procedures are to be used. Recommended field observations include color, odor, clarity, presence of floatables and deposits, and rate of flow, in addition to chemical measurements for fluoride, potassium, ammonia, and detergents (or fluorescence).

Collection of Samples and Field Analyses

All outfalls should be evaluated, not just those larger than a certain size. Lalor (1994) found that the smallest outfalls were typically the most contaminated because they were likely to be associated with creek-side small automotive businesses that improperly disposed of their wastes through small pipes. Figure 6.113 illustrates the simple sample collection methods used. The creeks are walked and all outfalls observed are evaluated. Generally, three-person crews are used, two walking the creek with waders, sampling equipment, and notebooks, while the third person drives the car to the next downstream meeting location (typically about 1/2 mile). It requires several (typically at least three) trips along a stream to find all the outfalls. Multiple sampling visits are also needed throughout the year to verify changing discharge conditions. Outfalls may be dry during some visits, but flowing during others.

We have found it to be much more convenient and efficient to collect samples in the field and return them to the laboratory where groups of samples can be evaluated together. Some simple field analyses are appropriate. Figure 6.114 shows a portable gas analyzer that can indicate explosive conditions, lack of oxygen, and the presence of H_2S. This is important from a safety standpoint in areas having little ventilation, and the H_2S can also be used to indicate sewage problems. Most of the field test kits examined during this research (and as summarized earlier in this chapter) would take much too long to conduct correctly and safely in the field.

Simple Data Evaluation Methods to Indicate Sources of Contamination

Negative Indicators Implying Contamination

Indicators of contamination (negative indicators) are clearly apparent visual or physical parameters indicating obvious problems and are readily observable at the outfall during the field screening activities. Relying only on these indicators can lead to an unacceptably high rate of false negatives and false positives and must therefore be supplemented with additional confirmatory methods. However, these indicators are easy to measure, are useful for indicating gross contamination, are easy to describe to nontechnical decision makers, and are therefore highly recommended as an important part of a field screening effort.

These observations are very important during the field survey because they are the simplest method of identifying grossly contaminated dry-weather outfall flows. The direct examination of outfall characteristics for unusual conditions of flow, odor, color, turbidity, floatables, deposits/stains, vegetation conditions, and damage to drainage structures is therefore an important part of these investigations. Table 6.35 presents a summary of these indicators, along with narratives of the descriptors to be selected in the field.

Figure 6.113 Collecting outfall samples for inappropriate discharge evaluations.

Figure 6.114 Portable gas analyzer for H_2S and explosive conditions.

Table 6.35 Interpretations of Physical Observation Parameters and Possible Associated Flow Sources

Odor — Most strong odors, especially gasoline, oils, and solvents, are likely associated with high responses on the toxicity screening test. Typical obvious odors include gasoline, oil, sanitary wastewater, industrial chemicals, decomposing organic wastes, etc.

sewage: smell associated with stale sanitary wastewater, especially in pools near outfall or septic system drainage.

sulfur ("rotten eggs"): industries that discharge sulfide compounds or organics (meat packers, canneries, dairies, etc.).

oil and gas: petroleum refineries or many facilities associated with vehicle maintenance or petroleum product storage.

rancid-sour: food preparation facilities (restaurants, hotels, etc.).

Color — Important indicator of inappropriate industrial sources. Industrial dry-weather discharges may be of any color, but dark colors, such as brown, gray, or black, are most common.

yellow: chemical plants, textile and tanning plants.

brown: meat packers, printing plants, metal works, stone and concrete, fertilizers, and petroleum refining facilities.

green: chemical plants, textile facilities.

red: meat packers or iron oxide from groundwater seeps, e.g., acid mine drainage.

gray: dairies, sewage.

Turbidity — Often affected by the degree of gross contamination. Dry-weather industrial flows with moderate turbidity can be cloudy, while highly turbid flows can be opaque. High turbidity is often a characteristic of undiluted dry-weather industrial discharges or soil erosion.

cloudy: sanitary wastewater, concrete or stone operations, fertilizer facilities, automotive dealers.

opaque: food processors, lumber mills, metal operations, pigment plants.

Floatable Matter — A contaminated flow may contain floating solids or liquids directly related to industrial, sanitary wastewater pollution, or agricultural feed lots. Floatables of industrial origin may include animal fats, spoiled food, oils, solvents, sawdust, foams, packing materials, or fuel.

oil sheen: petroleum refineries or storage facilities and vehicle service facilities.

sewage: sanitary wastewater.

Deposits and Stains — Refers to any type of coating near the outfall and are usually of a dark color. Deposits and stains often will contain fragments of floatable substances. These situations are illustrated by the grayish-black deposits that contain fragments of animal flesh and hair which often are produced by leather tanneries, or the white crystalline powder which commonly coats outfalls due to nitrogenous fertilizer wastes.

sediment: construction site or agricultural soil erosion.

oily: petroleum refineries or storage facilities, vehicle service facilities, and large parking lot runoff.

Vegetation — Vegetation surrounding an outfall may show the effects of industrial pollutants. Decaying organic materials coming from various food product wastes would cause an increase in plant life, while the discharge of chemical dyes and inorganic pigments from textile mills could noticeably decrease vegetation. It is important not to confuse the adverse effects of high stormwater flows on vegetation with highly toxic dry-weather intermittent flows.

excessive growth: food product facilities, sewage, or agricultural operations.

inhibited growth: high stormwater flows, beverage facilities, printing plants, metal product facilities, drug manufacturing, petroleum facilities, vehicle service facilities and automobile dealers, pesticide spraying.

Damage to Outfall Structures — Another readily visible indication of industrial contamination. Cracking, deterioration, and spalling of concrete or peeling of surface paint, occurring at an outfall are usually caused by severely contaminated discharges, usually of industrial origin. These contaminants are usually very acidic or basic in nature. Primary metal industries have a strong potential for causing outfall structural damage because their batch dumps are highly acidic. Poor construction, hydraulic scour, and old age may also adversely affect the condition of the outfall structure.

concrete cracking: industrial flows.

concrete spalling: industrial flows.

peeling paint: industrial flows.

metal corrosion: industrial flows.

From Pitt et al. 1993.

This method does not allow quantifiable estimates of the flow components, and it will very likely result in many incorrect negative determinations (missing outfalls that have important levels of contamination). These simple characteristics are most useful for identifying gross contamination. Only the most significant outfalls and drainage areas would therefore be recognized from this method. The other methods, requiring chemical determinations, can be used to quantify the flow contributions and to identify the less obviously contaminated outfalls.

Indications of intermittent flows (especially stains or damage to the structure of the outfall) could indicate serious illegal toxic pollutant entries into the storm drainage system that will be very difficult to detect and correct. Highly irregular dry-weather outfall flow rates or chemical characteristics could indicate industrial or commercial inappropriate entries into the storm drain system.

Correlation tests were conducted to identify relationships between outfalls that were known to have severe contamination problems and the negative indicators (Lalor 1994). Pearson correlation tests indicated that high turbidity (lack of clarity) and odors appeared to be the most useful physical indicators of contamination when contamination was defined by toxicity and the presence of detergents. Lack of clarity best indicated the presence of detergents, with an 80% correlation. As noted later, the detergent test was the single most useful of the chemical tests for distinguishing between contaminated and uncontaminated flows. The Pearson correlation tests also showed that noticeable odor was the best indicator of toxicity, with a 77% correlation. There is no theoretical connection between the physical indicators and these problems. High turbidity was noted in 74% of the contaminated source flow samples. This represented a 26% false negative rate (indication of no contamination when contamination actually exists), if one relied on turbidity alone as an indicator of contamination. High turbidity was noted in only 5% of the uncontaminated source flow samples. This represents the rate of false positives (indication of contamination when none actually exists) when relying on turbidity alone. Noticeable odor was indicated in 67% of flow samples from contaminated sources, but in none of the flow samples from uncontaminated sources. This translates to 37% false negatives, but no false positives. Obvious odors identified included gasoline, oil, sanitary wastewater, industrial chemicals or detergents, decomposing organic wastes, etc. A 65% correlation was also found to exist between color and Microtox toxicity. Color is an important indicator of inappropriate industrial sources, but it was also associated with some of the residential and commercial flow sources. Color was noted in 100% of the flow samples from contaminated sources, but it was also noted in 40% of the flow samples from uncontaminated sources. This represents 60% false positives, but no false negatives. Finally, a 63% correlation between the presence of sediments (assessed as settleable solids in the collection bottles of these source samples) and Microtox toxicity was also found. Sediments were noted in 34% of the samples from contaminated sources and in none of the samples from uncontaminated sources.

False negatives are more of a concern than a reasonable number of false positives when working with a screening methodology. Screening methodologies are used to direct further, more detailed investigations. False positives would be discarded after further investigation. However, a false negative during a screening investigation results in the dismissal of a problem outfall for at least the near future. Missed contributors to stream contamination may result in unsatisfactory in-stream results following the application of costly corrective measures elsewhere.

The method of using physical characteristics to indicate contamination in outfall flows does not allow quantifiable estimates of the flow components and, if used alone, will likely result in many incorrect determinations, especially false negatives. However, these simple characteristics are most useful for identifying gross contamination: only the most significantly contaminated outfalls and drainage areas would therefore be recognized using this method.

Detergents as Indicators of Contamination

Results from Mann–Whitney U tests (at the $\alpha = 0.05$ confidence level) indicated that samples from any of the dry-weather flow sources could be correctly classified as clean or contaminated based only on the measured value of any one of the following parameters: detergents, color, or conductivity (Lalor 1994). Color and high conductivity were present in samples from clean sources as well as contaminated sources, but their levels of occurrence were significantly different between the two groups. If samples from only one source were expected to make up outfall flows, the level of color or conductivity could be used to distinguish contaminated outfalls from clean outfalls. However, since multisource flows occur, measured levels of color or conductivity could fall within acceptable levels because of dilution, even though a contaminating source was contributing to the flow. Detergents (anionic surfactants), on the other hand, can be used to distinguish between clean and contaminated outfalls simply by their presence or absence, using a detection limit of 0.06 mg/L. All samples analyzed from contaminated sources contained detergents in excess of this amount (with the exception of three septage samples collected from homes discharging only toilet flushing water). No clean source samples were found to contain detergents. Contaminated sources would be detected in mixtures with uncontaminated waters if they made up at least 10% of the mixture.

The HACH detergents test was used during these analyses and was found to work very well. Unfortunately, this test uses a large amount of benzene for sample extractions and so great care is needed with the analysis and waste disposal. Only the most highly trained analysts, understanding the dangers of using benzene, should be allowed to use this test. An alternative method examined by CHEMetrics uses relatively small amounts of chloroform (well contained) for sample extractions and is therefore much safer, although care is also needed during the test and in disposal of waste. However, this method has a poorer detection limit (about 0.15 mg/L) than the HACH method, leading to less sensitivity (and possible false negatives).

Because of the hazardous problems associated with using these simple detergent (anionic surfactant) tests, we have investigated numerous alternative, but related, tests. Standard tests for boron are relatively simple, safe, and sensitive. Historically, boron was an important component in laundry detergents and tests were conducted to see if this analysis would be a suitable substitute for the detergent tests. Unfortunately, boron appears to have been replaced in most U.S. detergents, as numerous tests of commercial laundry products found little boron. In addition, boron tests of sewage mixtures and from numerous mixed wastewaters from throughout the country also indicated little boron. Fluorescence of test waters, using an extremely sensitive, but expensive, fluorometer (Turner 10-AU), was also evaluated, but with mixed results. The analyses of sewage and detergents found highly variable fluorescence values because of the highly variable amounts of fabric whiteners found in detergents. However, it is possible to use fluorescence as a good presence/absence test, like the initial detergent evaluations. The previous discussion of optical brighteners (as a field test kit) indicated the potential usefulness of this method, but more work is needed to determine its sensitivity. As indicated later, more sophisticated tests for detergent components (LAS and perfumes, especially) have been successfully used as sewage tracers in many waters, but these analyses require expensive and time-consuming HPLC analyses.

Simple Checklist for Major Flow Component Identification

Table 6.36 is a simplification of the analysis strategy to separate the major nonstormwater discharge sources for areas having no industrial activity. The first indicator is the presence or absence of flow. If no dry-weather flow exists at an outfall, then indications of intermittent flows must be investigated. Specifically, stains, deposits, odors, unusual stream-side vegetation conditions, and outfall structural damage can all indicate intermittent nonstormwater flows. However, multiple visits to outfalls over long time periods are needed to confirm that only stormwater flows occur. The following paragraphs summarize the rationale used to distinguish between treated potable water and sanitary wastewater, the two most common dry-weather flow sources in storm drainage systems in residential and commercial areas.

Table 6.36 Simplified Checklist to Identify Residential Area Non-Stormwater Flow Sources

1. Flow? If yes, go to 2; if no, go to 3.
2. Fluorides (or different hardness)? If yes, probably treated water (may be contaminated), go to 4; if no, then untreated natural water (probably uncontaminated), or untreated industrial water (may be toxic).
3. Check for intermittent dry-weather flow signs (may be contaminated). If yes, recheck outfall at later date; if no, then not likely a significant non-stormwater source.
4. Surfactants (or fluorescence)? If yes, may be sanitary wastewater, laundry water, or other wash water (may be pathogenic, or nuisance), go to 5; if no, then may be domestic water line leak, irrigation runoff, or rinse water (probably not a contaminated non-stormwater source, but may be a nuisance).
5. Elevated potassium (or ammonia)? If yes, then likely sanitary wastewater source (pathogenic); if no, likely wash water (probably not a contaminated non-stormwater source, but may be a nuisance).

From Pitt et al. 1993.

Treated Potable Water — A number of tracer parameters may be useful for distinguishing treated potable water from natural waters:

- Major ions or other chemical/physical characteristics of the flow components can vary substantially, depending on whether the water supply sources are groundwater or surface water, and whether the sources are treated or not. Specific conductance may also serve as an indicator of the major water source.
- Fluoride can often be used to separate treated potable water from untreated water sources. This latter group may include local springs, groundwater, regional surface flows, or nonpotable industrial waters. If the treated water has no fluoride added, or if the natural water has fluoride concentrations close to potable water fluoride concentrations, then fluoride may not be an appropriate indicator. Water from treated water supplies (that test positive for fluorides or other suitable tracers) can be relatively uncontaminated (domestic water line leakage or irrigation runoff), or it may be heavily contaminated. If the drainage area has industries that have their own water supplies (quite rare for most urban drainage areas), further investigations are needed to check for industrial nonstormwater discharges. Toxicity screening methods would be very useful in areas known to have commercial or industrial activity, or to check for intermittent residential area discharges of toxicants. Fluoride can be very high in some commercial wash waters and industrial wastewaters.
- Hardness can also be used as an indicator if the potable water source and the baseflow are from different water sources. An example would be if the baseflow is from hard groundwater and the potable water is from softer surface supplies.
- If the concentration of chlorine is high, then a major leak of disinfected potable water is probably close to the outfall. Because of the rapid loss of chlorine in water (especially if some organic contamination is present), it is not a good parameter for quantifying the amount of treated potable water observed at the outfall.

Water from potable water supplies (that test positive for fluorides, or other suitable tracers) can be relatively uncontaminated (domestic water line leakage or irrigation runoff) or heavily contaminated (sanitary wastewater).

Sanitary Wastewaters — In areas containing no industrial or commercial sources, sanitary wastewater is probably the most important dry-weather source of storm drain flows. In addition, septic systems often do not operate properly and might be a significant source of contamination in rural areas. The following parameters can be used for quantifying the sanitary wastewater components of the treated domestic water portion:

- Surfactant (detergent) analyses may be useful in determining the presence of sanitary wastewaters, as noted previously. However, surfactants present in water originating from potable water sources could indicate sanitary wastewaters, laundry wastewaters, car washing wastewater, or any other waters containing surfactants. If surfactants are not present, then the potable water could be relatively uncontaminated (domestic water line leaks or irrigation runoff).

- The presence of fabric whiteners (as measured by fluorescence) can also be used in distinguishing laundry and sanitary wastewaters.
- Sanitary wastewaters often exhibit predictable trends during the day in flow and quality. In order to maximize the ability to detect direct sanitary wastewater connections into the storm drainage system, it would be best to survey the outfalls during periods of highest sanitary wastewater flows (mid to late morning hours).
- The ratio of surfactants to ammonia or potassium concentrations may be an effective indicator of the presence of sanitary wastewaters or septic tank effluents. If the surfactant concentrations are high, but the ammonia and potassium concentrations are low, the contaminated source may be laundry wastewaters. Conversely, if ammonia, potassium, and surfactant concentrations are all high, sanitary wastewater is the likely source. Some researchers have reported low surfactants in septic tank effluents. Therefore, if surfactants are low but potassium and ammonia are both high, septic tank effluent may be present. However, research in the Birmingham, AL, area found high surfactant concentrations in septic tank effluent, further stressing the need to obtain local characterization data for potential contaminating sources.
- Obviously, odor and other physical appearances such as turbidity, coarse and floating "tell-tale" solids, foaming, color, and temperature would also be very useful in distinguishing sanitary wastewater from wash water or laundry wastewater sources, as noted previously. However, these indicators may not be very obvious for small levels of sanitary wastewater contamination.

Flowchart for Most Significant Flow Component Identification

A further refinement of the above checklist is the flowchart shown on Figure 6.115. This flow chart describes an analysis strategy that may be used to identify the major component of dry-weather flow samples in residential and commercial areas. This method does not attempt to distinguish among all potential sources of dry-weather flows identified earlier, but rather the following four major groups of flow are identified: (1) tap waters (including domestic tap water, irrigation water, and rinse water), (2) natural waters (spring water and shallow groundwater), (3) sanitary wastewaters (sanitary sewage and septic tank discharge), and (4) wash waters (commercial laundry waters, commercial car wash waters, radiator flushing wastes, and plating bath wastewaters). The use of this method would not only allow outfall flows to be categorized as contaminated or uncontaminated, but would also allow outfalls carrying sanitary wastewaters to be identified. These outfalls could then receive the highest priority for further investigation leading to source control. This flowchart was designed for use in residential and/or commercial areas only. Investigations in industrial or industrial/commercial land use areas must be approached in an entirely different manner.

In residential and/or commercial areas, all outfalls should be located and examined. The first indicator is the presence or absence of dry-weather flow. If no dry-weather flow exists at an outfall, indications of intermittent flows must be investigated. Specifically, stains, deposits, odors, unusual stream-side vegetation conditions, and damage to outfall structures can all indicate intermittent nonstormwater flows. However, frequent visits to outfalls over long time periods, or the use of other monitoring techniques, may be needed to confirm that only stormwater flows occur. If intermittent flow is not indicated, the outfall probably does not have a contaminated nonstormwater source. The other points on the flowchart serve to indicate if a major contaminating source is present, or if the water is uncontaminated. Component contributions cannot be quantified using this method, and only the "most contaminated" type of source present will be identified.

If dry-weather flow exists at an outfall, then the flow should be sampled and tested for detergents. If detergents are not present, the flow is probably from a noncontaminated nonstormwater source. The lower limit of detection for detergent should be about 0.06 mg/L.

If detergents are not present, fluoride levels can be used to distinguish between flows with treated water sources and flows with natural sources in communities where water supplies are fluoridated and natural fluoride levels are low. In the absence of detergents, high fluoride levels

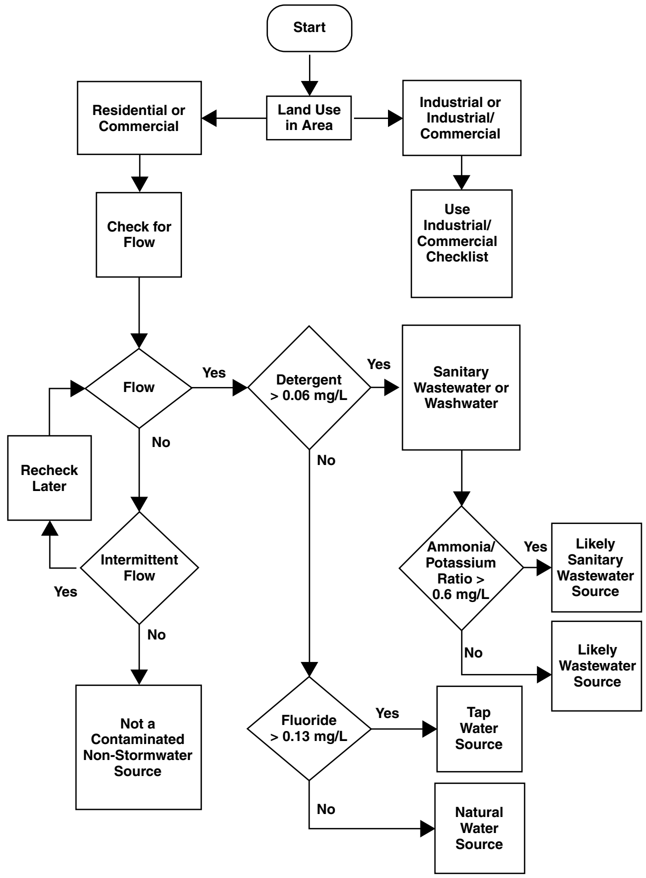

Figure 6.115 Simple flowchart method to identify significant contaminating sources. (From Lalor, M. *An Assessment of Non-Stormwater Discharges to Storm Drainage Systems in Residential and Commercial Land Use Areas.* Ph.D. dissertation. Department of Civil and Environmental Engineering. Vanderbilt University. 1994. With permission.)

would indicate a potable water line leak, irrigation water, or wash/rinse water. Low fluoride levels would indicate waters originating from springs or shallow groundwater. Based on the flow source samples tested in this research (Table 6.34), fluoride levels above 0.13 mg/L would most likely indicate that a tap water source was contributing to the dry-weather flow in the Birmingham, AL, study area.

If detergents are present, the flow is probably from a contaminated nonstormwater source, as indicated on Table 6.34. The ratio of ammonia to potassium can be used to indicate whether or not the source is sanitary wastewater. Ammonia/potassium ratios greater than 0.60 would indicate likely sanitary wastewater contamination. Ammonia/potassium ratios were above 0.9 for all septage and sewage samples collected in Birmingham (values ranged from 0.97 to 15.37, averaging 2.55). Ammonia/potassium ratios for all other samples containing detergents were below 0.7, ranging from 0.00 to 0.65, averaging 0.11. One radiator waste sample had an ammonia/potassium ratio of 0.65.

Noncontaminated samples collected in Birmingham had ammonia/potassium ratios ranging from 0.00 to 0.41, with a mean value of 0.06 and a median value of 0.03. Using the mean values for noncontaminated samples (0.06) and sanitary wastewaters (2.55), flows comprised of mixtures containing at least 25% sanitary wastes with the remainder of the flow from uncontaminated sources would likely be identified as sanitary wastewaters using this method. Flows containing a smaller percentage of contributions from sanitary wastewaters might be identified as having a wash water source, but would not be identified as uncontaminated.

General Matrix Algebra Methods to Indicate Sources of Contamination through Fingerprinting

Other approaches can also be used to calculate the source components of mixed outfall flows. One approach is the use of matrix algebra to simultaneously solve a series of chemical mass balance equations. This method can be used to predict the most likely flow source, or sources, making up an outfall sample. It is possible to estimate the outfall source flow components using a set of simultaneous equations. The number of unknowns should equal the number of equations available, resulting in a square matrix. If there are seven likely source categories, then there should be seven tracer parameters used. If there are only four possible sources, then only the four most efficient tracer parameters should be used. Only tracers that are linearly related to mixture components can be used. As an example, pH cannot be used in these equations, because it is not additive.

Further site-specific statistical analyses may be needed to rank the usefulness of the tracers for distinguishing different flow sources. As an example, chlorine is generally not useful for these analyses because the concentration variability within many source categories is high (it is also not a conservative parameter). Chlorine may still be a useful parameter, but only to identify possible large potable water line leaks. Another parameter having problems for most situations is pH. The variation of pH between sources is very low (they are all very similar). pH may still be useful to identify industrial wastewater problems, but it cannot be used to quantify flow components. Toxicity is another parameter used during this research that was found not to be linearly additive.

This method estimates flow contributions from various sources using a "receptor model," based on a set of chemical mass balance equations. Such models, which assess the contributions from various sources based on observations at sampling sites (the receptors), have been applied to the investigation of air pollutant sources for many years (Lee et al. 1993; Cooper and Watson 1980). The characteristic "signatures" of the different types of sources, as identified in the library of source flow data, allow the development of a set of mass balance equations. These equations describe the measured concentrations in an outfall's flow as a linear combination of the contributions from the different potential sources. A major requirement for this method is the physical and chemical characterization of waters collected directly from potential sources of dry-weather flows (the "library"). This allows concentration patterns (fingerprints) for the parameters of interest to be established for each type of source. Theoretically, if these patterns are different for each source, the observed concentrations at the outfall would be a linear combination of the concentration patterns from the different component sources, each weighted by a source strength term (m_n). This source strength term would indicate the fraction of outfall flow originating from each likely source. By measuring a number of parameters equal to, or greater than, the number of potential source types, the source strength term could be obtained by solving a set of chemical mass balance equations of the type:

$$C_p = \sum_n m_n x_{pn}$$

where C_p is the concentration of parameter p in the outfall flow and x_{pn} is the concentration of parameter p in source type n.

As an example of this method, consider eight possible flow sources and eight parameters, as presented in Table 6.37. The number of parameters evaluated for each outfall must equal the number of probable dry-weather flow sources in the drainage area. Mathematical methods are available which provide for the solution of over-specified sets of equations (more equations than unknowns), but these are not addressed here.

The selection of parameters for measurement should reflect evaluated parameter usefulness. Evaluation of the Mann–Whitney U Test results (Lalor 1994) suggested the following groupings of parameters, ranked by their usefulness for distinguishing between all the types of flow sources sampled in Birmingham, AL:

- First set (most useful): potassium and hardness
- Second set: fluorescence, conductivity, fluoride, ammonia, detergents, and color
- Third set (least useful): chlorine

If parameter variations within the sources are not accounted for, the equations would take the form presented in Table 6.38. Here, the x terms, representing parameter concentrations within the specified source, have been replaced with the mean concentrations noted in the source library. After measured values are substituted into the equations for parameter concentrations in the outfall flow (C_p), this set of simultaneous equations can be solved using matrix algebra. The use of mean concentration values in the equation set was evaluated by entering the potential dry-weather flow source samples from Birmingham as unknowns (as if they were outfall samples) and solving for fractions of flow (the m terms in Table 6.38). This exercise resulted in four false negatives (6%) and 27 false positives (73%). The results of these simple preliminary tests indicated that there was too much variation of parameter concentrations within the various source types to allow them to be adequately characterized by simple use of the mean concentrations alone. Another method, recognizing variations in source flow characteristics in a Monte Carlo model, is presented by Lalor (1994). Both of these methods listed the likely multiple contaminating sources and estimated their relative contributions. Unfortunately, confirmation testing indicated inaccurate results much of the time, implying the greater usefulness of the simpler methods described previously. However, these matrix algebra methods may be very useful in other situations or locations and should be investigated as part of a local screening project to identify inappropriate discharges to storm drainage.

There are numerous other statistical analysis methods suitable for identifying sources of flows. Salau et al. (1997) present a review of several advanced statistical methods also derived from air pollution source identification research (see Chapter 7 for illustrations from his paper). Principal component analysis and hierarchical cluster analysis are shown as tools that can identify common sources of contamination by examining a set of well-selected tracer compounds (in northwest Mediterranean marine sediments in their example). These are used to develop the alternating least squares approach, similar to Lalor's (1994) use of these same techniques to identify the best parameters for the simultaneous equation solutions described above.

Table 6.37 Set of Chemical Mass Balance Equations

	Source 1		Source 2		Source 3		Source 4		Source 5		Source 6		Source 7		Source 8		Outfall
Parameter 1:	(m1)(x11)	+	(m2)(x12)	+	(m3)(x13)	+	(m4)(x14)	+	(m5)(x15)	+	(m6)(x16)	+	(m7)(x17)	+	(m8)(x18)	=	C1
Parameter 2:	(m1)(x21)	+	(m2)(x22)	+	(m3)(x23)	+	(m4)(x24)	+	(m5)(x25)	+	(m6)(x26)	+	(m7)(x27)	+	(m8)(x28)	=	C2
Parameter 3:	(m1)(x31)	+	(m2)(x32)	+	(m3)(x33)	+	(m4)(x34)	+	(m5)(x35)	+	(m6)(x36)	+	(m7)(x37)	+	(m8)(x38)	=	C3
Parameter 4:	(m1)(x41)	+	(m2)(x42)	+	(m3)(x43)	+	(m4)(x44)	+	(m5)(x45)	+	(m6)(x46)	+	(m7)(x47)	+	(m8)(x48)	=	C4
Parameter 5:	(m1)(x51)	+	(m2)(x52)	+	(m3)(x53)	+	(m4)(x54)	+	(m5)(x55)	+	(m6)(x56)	+	(m7)(x57)	+	(m8)(x58)	=	C5
Parameter 6:	(m1)(x61)	+	(m2)(x62)	+	(m3)(x63)	+	(m4)(x64)	+	(m5)(x65)	+	(m6)(x66)	+	(m7)(x67)	+	(m8)(x68)	=	C6
Parameter 7:	(m1)(x71)	+	(m2)(x72)	+	(m3)(x73)	+	(m4)(x74)	+	(m5)(x75)	+	(m6)(x76)	+	(m7)(x77)	+	(m8)(x78)	=	C7
Parameter 8:	(m1)(x81)	+	(m2)(x82)	+	(m3)(x83)	+	(m4)(x84)	+	(m5)(x85)	+	(m6)(x86)	+	(m7)(x87)	+	(m8)(x88)	=	C8

Equations of the form $C_p = \sum_n m_n x_{pn}$

where: C_p = the concentration of parameter p in the outfall flow

m_n = the fraction of flow from source type n

x_{pn} = the mean concentration of parameter p in source type n

From Lalor, M. *An Assessment of Non-Stormwater Discharges to Storm Drainage Systems in Residential and Commercial Land Use Areas.* Ph.D. dissertation. Department of Civil and Environmental Engineering. Vanderbilt University. 1994. With permission.

Once sources are identified, it is important to confirm their source and to ensure that corrective action is undertaken. Figures 6.116 and 6.117 show TV surveying being conducted in Boston to confirm the likely source of inappropriate discharges. Normally, the TV camera is remotely operated and pulled through small pipes (Figure 6.116). However, in the coastal area and in large pipes, crews were required to conduct the surveys manually (Figure 6.117).

Emerging Tools for Identifying Sources of Discharges

Coprostanol and Other Fecal Sterol Compounds Utilized as Tracers of Contamination by Sanitary Sewage

A more likely indicator of human wastes than fecal coliforms and other "indicator" bacteria may be the use of certain molecular markers, specifically the fecal sterols, such as coprostanol and epicoprostanol (Eaganhouse et al. 1988). However, these compounds are also discharged by other carnivores (especially dogs) in a drainage. A number of research projects have used these compounds to investigate the presence of sanitary sewage contamination. The most successful application may be associated with sediment analyses instead of water analyses. As an example, water analyses of coprostanol are difficult due to the typically very low concentrations found, although the concentrations in many sediments are quite high and much easier to quantify. Unfortunately, the long persistence of these compounds in the environment easily confuses recent contamination with historical or intermittent contamination.

Particulates and sediments collected from coastal areas in Spain and Cuba receiving municipal sewage loads were analyzed by Grimalt et al. (1990) to determine the utility of coprostanol as a chemical marker of sewage contamination. Coprostanol cannot by itself be attributed to fecal matter inputs. However, relative contributions of steroid components can be useful indicators. When the relative concentrations of coprostanol and coprostanone are higher than their 5α epimers, or more realistically, other sterol components of background or natural occurrence, they can provide useful information.

Sediment cores from Santa Monica Basin, CA, and effluent from two local municipal wastewater discharges were analyzed by Venkatesan and Kaplan (1990) for coprostanol to determine the degree of sewage addition to sediment. Coprostanols were distributed throughout the basin sediments in association with fine particles. Some stations contained elevated levels, either due to their proximity to outfalls or because of preferential advection of fine-grained sediments. A noted decline of coprostanols relative to total sterols from outfalls seaward indicated dilution of sewage by biogenic sterols.

Other chemical compounds have been utilized for sewage tracer work. Saturated hydrocarbons with 16 to 18 carbons, and saturated hydrocarbons with 16 to 21 carbons, in addition to coprostanol, were chosen as markers for sewage in water, particulate, and sediment samples near the Cocoa, FL, domestic wastewater treatment plant (Holm et al. 1990). The concentration of the markers was highest at points close to the outfall pipe and diminished with distance. However, the concentration of C16 to C21 compounds was high at a site 800 m from the outfall, indicating that these compounds were unsuitable markers for locating areas exposed to the sewage plume. The concentrations for the other markers were very low at this station.

The range of concentrations of coprostanol found in sediments and mussels of Venice, Italy, were reported by Sherwin et al. (1993). Raw sewage is still discharged directly into the Venice lagoon. Coprostanol concentrations were determined in sediment and mussel samples from the lagoon using gas chromatography/mass spectroscopy. Samples were collected in interior canals and compared to open-bay concentrations. Sediment concentrations ranged from 0.2 to 41.0 μg/g (dry weight). Interior canal sediment samples averaged 16 μg/g compared to 2 μg/g found in open-bay sediment samples. Total coprostanol concentrations in mussels ranged from 80 to 620 ng/g (wet weight). No mussels were found in the four most polluted interior canal sites.

Table 6.38 Chemical Mass Balance Equations with Parameter Means

Parameters	Spring Water 1	Ground-Water 2	Tap Water 3	Irrigation Water 4	Sanitary Sewage 5	Septic Tank 6	Car Wash 7	Laundry Water 8	Unknown Sample
Potassium	(m1)(0.73)	+ (m2)(1.19)	+ (m3)(1.55)	+ (m4)(6.08)	+ (m5)(5.97)	+ (m6)(18.82)	+ (m7)(42.69)	+ (m8)(3.48)	= (1)(C_p)
Hardness	(m1)(240)	+ (m2)(27)	+ (m3)(49)	+ (m4)(40)	+ (m5)(143)	+ (m6)(57)	+ (m7)(157)	+ (m8)(36)	= (1)(C_p)
Fluorescence	(m1)(6.8)	+ (m2)(29.9)	+ (m3)(4.6)	+ (m4)(214)	+ (m5)(251)	+ (m6)(382)	+ (m7)(1190)	+ (m8)(1024)	= (1)(C_p)
Conductivity	(m1)(301)	+ (m2)(51)	+ (m3)(112)	+ (m4)(105)	+ (m5)(420)	+ (m6)(502)	+ (m7)(485)	+ (m8)(563)	= (1)(C_p)
Fluoride	(m1)(0.03)	+ (m2)(0.06)	+ (m3)(0.97)	+ (m4)(0.90)	+ (m5)(0.76)	+ (m6)(0.93)	+ (m7)(12.3)	+ (m8)(32.82)	= (1)(C_p)
Ammonia	(m1)(0.01)	+ (m2)(0.24)	+ (m3)(0.03)	+ (m4)(0.37)	+ (m5)(9.92)	+ (m6)(87.21)	+ (m7)(0.24)	+ (m8)(0.82)	= (1)(C_p)
Detergents	(m1)(0.00)	+ (m2)(0.00)	+ (m3)(0.00)	+ (m4)(0.00)	+ (m5)(1.50)	+ (m6)(3.27)	+ (m7)(49.00)	+ (m8)(26.90)	= (1)(C_p)
Color	(m1)(0.0)	+ (m2)(8.0)	+ (m3)(0.0)	+ (m4)(10.0)	+ (m5)(37.9)	+ (m6)(70.6)	+ (m7)(221.5)	+ (m8)(46.7)	= (1)(C_p)

Equations of the form $C_p = \sum_n m_n x_{pn}$

where: C_p = the concentration of parameter p in the outfall flow

m_n = the fraction of flow from source type n

x_{pn} = the mean concentration of parameter p in source type n

From Lalor, M. *An Assessment of Non-Stormwater Discharges to Storm Drainage Systems in Residential and Commercial Land Use Areas.* Ph.D. dissertation. Department of Civil and Environmental Engineering. Vanderbilt University. 1994. With permission.

Figure 6.116 Remotely operated TV camera surveys of storm sewers in Boston to locate inappropriate discharges.

Figure 6.117 Manual surveys conducted in Boston in large tidally influenced storm drains.

Nichols et al. (1996) also examined coprostanol in stormwater and the sea-surface microlayer to distinguish human vs. nonhuman sources of contamination. Other steroid compounds in sewage effluent were investigated by Routledge et al. (1998) and Desbrow et al. (1998), who both examined estrogenic chemicals. The most commonly found were 17β-estradiol and estrone, which were detected at concentrations in the tens of nanograms per liter range. These were identified as estrogenic through a toxicity identification and evaluation approach, where sequential separations and analyses identified the sample fractions causing estrogenic activity using a yeast-based estrogen screen. GC/MS was then used to identify the specific compounds.

Estimating Potential Sanitary Sewage Discharges into Storm Drainage and Receiving Waters Using Detergent Tracer Compounds

As described above, detergent measurements (using methylene blue active substance, MBAS, test methods) were the most successful individual tracers to indicate contaminated water in storm sewerage dry-weather flows. Unfortunately, the MBAS method uses hazardous chloroform for an extraction step. Different detergent components, especially linear alkylbenzene sulfonates (LAS) and linear alkylbenzenes (LAB), have also been tried to indicate sewage dispersal patterns in receiving waters. Boron, a major historical ingredient of laundry chemicals, can also potentially be used. Boron has the great advantage of being relatively easy to analyze using portable field test kits, while LAS requires chromatographic equipment. LAS can be measured using HPLC with fluorescent detection, after solid-phase extraction, to very low levels. Fujita et al. (1998) developed an efficient enzyme-linked immunosorbent assay (ELISA) for detecting LAS at levels from 20 to 500 μg/L.

LAS from synthetic surfactants (Terzic and Ahel 1993) which degrade rapidly, as well as nonionic detergents (Zoller et al. 1991) which do not degrade rapidly, have been utilized as sanitary sewage markers. LAS was quickly dispersed from wastewater outfalls except in areas where wind was calm. In these areas, LAS concentrations increased in fresh water but were unaffected in saline water. After time, the lower alkyl groups were mostly found, possibly as a result of degradation or settling of longer alkyl chain compounds with sediments. Chung et al. (1995) also describe the distribution and fate of LAS in an urban stream in Korea. They examined different LAS compounds having carbon ratios of C12 and C13 compared to C10 and C11, plus ratios of phosphates to MBAS and the internal to external isomer ratio (I/E) as part of their research. González-Mazo et al. (1998)

examined LAS in the Bay of Cádiz off the southwest coast of Spain. They found that LAS degrades rapidly. Fujita et al. (1998) found that complete biodegradation of LAS requires several days and is also strongly sorbed to particulates. In areas close to shore and near the untreated wastewater discharges, there was significant vertical stratification of LAS: the top 3 to 5 mm of water had LAS concentrations about 100 times greater than those found at 0.5 m.

Zeng and Vista (1997) and Zeng et al. (1997) describe a study off San Diego where LAB was measured, along with polycyclic aromatic hydrocarbons (PAHs) and aliphatic hydrocarbons (AHs) to indicate the relative pollutant contributions of wastewater from sanitary sewage, nonpoint sources, and hydrocarbon combustion sources. They developed and tested several indicator ratios (alkyl homologue distributions and parent compound distributions) and examined the ratios of various PAHs (such as phenanthrene to anthracene, methylphenanthrene to phenanthrene, fluoranthene to pyrene, and benzo(a)anthracene to chrysene) as tools for distinguishing these sources. They concluded that LABs are useful tracers of domestic waste inputs to the environment due to their limited sources. They also describe the use of the internal to external isomer ratio (I/E) to indicate the amount of biodegradation that may have occurred to the LABs. They observed concentrations of total LABs in sewage effluent of about 3 μg/L, although previous researchers have seen concentrations of about 150 μg/L in sewage effluent from the same area.

The fluorescent properties of detergents have also been used as tracers by investigating the fluorescent whitening agents (FWAs), as described by Poiger et al. (1996) and Kramer et al. (1996). HPLC with fluorescence detection was used in these studies to quantify very low concentrations of FWAs. The two most frequently used FWAs in household detergents (DSBP and DAS 1) were found at 7 to 21 μg/L in primary sewage effluent and at 3 to 9 μg/L in secondary effluent. Raw sewage contains about 10 to 20 μg/L FWAs. The removal mechanisms in sewage treatment processes is by adsorption to activated sludge. The type of FWAs varies from laundry applications to textile finishing and paper production, making it possible to identify sewage sources. The FWAs were found in river water at 0.04 to 0.6 μg/L. The FWAs are not easily biodegradable, but they are readily photodegraded. Photodegradation rates have been reported to be about 7% for DSBP and 71% for DAS 1 in river water exposed to natural sunlight, after 1-hour exposure. Subsequent photodegradation is quite slow.

Other Compounds Found in Sanitary Sewage That May Be Used for Identifying Contamination by Sewage

Halling-Sørensen et al. (1998) detected numerous pharmaceutical substances in sewage effluents and in receiving waters. Their work addressed human health concerns of these low-level compounds that can enter downstream drinking water supplies. However, the information might also be used to help identify sewage contamination. Most of the research has focused on clofibric acid, a chemical used in cholesterol-lowering drugs. It has been found in concentrations ranging from 10 to 165 ng/L in a Berlin drinking water sample. Other drugs commonly found include aspirin, caffeine, and ibuprofen. Current FDA guidance mandates that the maximum concentration of a substance or its active metabolites at the point of entry into the aquatic environment be less than 1 μg/L (Hun 1998).

Caffeine has been used as an indicator of sewage contamination by several investigators (Shuman and Strand 1996). The King County, WA, Water Quality Assessment Project is examining the impacts of CSOs on the Duwamish River and Elliott Bay. They are using both caffeine (representing dissolved CSO constituents) and coprostanol (representing particulate-bound CSO constituents), in conjunction with heavy metals and conventional analyses, to help determine the contribution of CSOs to the river. The caffeine is unique to sewage, while coprostanol is from both humans and carnivorous animals and is therefore also in stormwater. They sampled upstream of all CSOs, but with some stormwater influences, 100 m upstream of the primary CSO discharge (but downstream of other CSOs), within the primary CSO discharge line, and 100 m downriver of the CSO discharge location. The relationship between caffeine and coprostanol was fairly consistent for the four sites (coprostanol was about 0.5 to 1.5 μg/L higher than caffeine). Similar patterns were found between the three metals, chromium was always the lowest and zinc was the highest. King County is also using clean transported mussels placed in the Duwamish River to measure the bioconcentration potential of metal and organic toxicants and the effects of the CSOs on mussel growth rates (after 6-week exposure periods). Paired reference locations are available near the areas of deployment, but outside the areas of immediate CSO influence. *U.S. Water News* (1998) also described a study in Boston Harbor that found caffeine at levels of about 7 μg/L in the harbor water. The caffeine content of regular coffee is about 700 mg/L, in contrast.

DNA Profiling to Measure Impacts on receiving water Organisms and to Identify Sources of Microorganisms in Stormwater

This rapidly emerging technique seems to have great promise in addressing a number of nonpoint source water pollution issues. Kratch (1997) summarized several investigations on cataloging the DNA of *E. coli* to identify their source in water. The procedure, developed at the Virginia Polytechnic Institute and State University, has been used in Chesapeake Bay. In one example, it was possible to identify a large wild animal population as the source of fecal coliform contamination of a shellfish bed, instead of suspected failing septic tanks. DNA patterns in fecal coliforms vary among animals and birds, and it is relatively easy to distinguish between human and nonhuman sources of the bacteria. However, some wild animals have DNA patterns that are not easily distinguishable. Some researchers question the value of *E. coli* DNA fingerprinting, believing that there is little direct relationship between *E. coli* and human pathogens. However, this method should be useful to identify the presence of sewage contamination in stormwater or in a receiving water.

One application of the technique, as described by Krane et al. (1999) of Wright State University, used randomly amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) generated profiles of naturally occurring crayfish. They found that changes in the underlying genetic diversity of these populations were significantly correlated with the extent to which they have been exposed to anthropogenic stressors. They concluded that this rapid and relatively simple technique can be used to develop a sensitive means of directly assessing the impact of stressors on ecosystems. These Wright State University researchers have also used the RAPD-PCR techniques on populations of snails, pill bugs, violets, spiders, earthworms, herring, and some benthic macroinvertebrates, finding relatively few obstacles in its use for different organisms. As noted above, other researchers have used DNA profiling techniques to identify sources of *E. coli* bacteria found in coastal waterways. It is possible that these techniques can be expanded to enable rapid detection of many different types of pathogens in receiving waters, and the most likely sources of these pathogens.

Stable Isotope Methods for Identifying Sources of Water

Stable isotopes had been recommended as an efficient method to identify illicit connections to storm sewerage. A demonstration was conducted in Detroit as part of the Rouge River project to identify sources of dry-weather flows in storm sewerage (Sangal et al. 1996). Naturally occurring stable isotopes of oxygen and hydrogen can be used to identify waters originating from different geographical sources (especially along a north–south gradient). Ma and Spalding (1996) discuss this approach by using stable isotopes to investigate recharge of groundwaters by surface waters. During water vapor transport from equatorial source regions to higher latitudes, depletion of heavy isotopes occurs with rain. Deviation from a standard relationship between deuterium and ^{18}O for a specific area indicates that the water has undergone additional evaporation. The ratio is also affected by seasonal changes. As discussed by Ma and Spalding (1996), the Platte River water is normally derived in part from snowmelt from the Rocky Mountains, while the groundwater in parts of Nebraska is mainly contributed from the Gulf air stream. The origins of these waters are sufficiently different and allow good measurements of the recharge rate of the surface water to the groundwater. In Detroit, Sangal et al. (1996) used differences in origin between the domestic water supply, local surface waters, and the local groundwater to identify potential sanitary sewage contributions to the separate storm sewerage. Rieley et al. (1997) used stable isotopes of carbon in marine organisms to distinguish the primary source of carbon being consumed (sewage sludge vs. natural carbon sources) in two deep sea sewage sludge disposal areas.

Stable isotope analyses would not be able to distinguish between sanitary sewage, industrial discharges, wash waters, and domestic water, as they all have the same origin. Nor would it be possible to distinguish sewage from local groundwaters if the domestic water supply was from the same local aquifer. This method works best for situations where the water supply is from a distant source and where separation of waters into separate flow components is not needed. It may be an excellent tool to study the effects of deep well injection of stormwater on deep aquifers having distant recharge sources (such as in the Phoenix area). Few laboratories can analyze for these stable isotopes, requiring shipping the samples and a long wait for the analytical results. Sangal et al. (1996) used Geochron Laboratories, in Cambridge, MA.

Dating of sediments using ^{137}Cs was described by Davis et al. (1997). Arsenic-contaminated sediments in the Hylebos Waterway in Tacoma, WA, could have originated from numerous sources, including a pesticide manufacturing facility, a rock-wool plant, steel slags, powdered metal plant, shipbuilding facilities, marinas and arsenic-based boat paints, and the Tacoma Smelter. Dating the sediments, combined with knowing the history of potential discharges and conducting optical and electron microscopic studies of the sediments, was found to be a powerful tool to differentiate the metal sources to the sediments.

Comparison of Parameters That Can Be Used for Identifying Inappropriate Discharges to Storm Drainage

In almost all cases, a suite of analyses is most suitable for effective identification of inappropriate discharges. An example was reported by Standley et al. (2000), where fecal steroids (including coprostanol), caffeine, consumer product fragrance materials, and petroleum and combustion by-products were used to identify wastewater treatment plant effluent, agricultural and feedlot runoff, urban runoff, and wildlife sources. They studied numerous individual sources of these wastes from throughout the United States. A research-grade mass spectrophotometer was used for the majority of the analyses in order to achieve the needed sensitivities, although much variability was found when using the methods in actual receiving waters affected by wastewater effluent. This sophisticated suite of analyses did yield much useful information, but the analyses are difficult to conduct and costly and may be suitable for special situations, but not for routine survey work.

Another series of tests examined several of these potential emerging tracer parameters, in conjunction with the previously identified parameters, during a project characterizing stormwater that had collected in telecommunication manholes, funded by Tecordia (previously Bellcore), AT&T, and eight regional telephone companies throughout the country (Pitt and Clark 1999). Numerous conventional constituents, plus major ions, and toxicants were measured, along with candidate tracers to indicate sewage contamination of this water. Boron, caffeine, coprostanol, *E. coli*, enterococci, fluorescence (using specific wavelengths for detergents), and a simpler test for detergents were evaluated, along with the use of fluoride, ammonia, potassium, and obvious odors and color. About 700 water samples were evaluated for all of these parameters, with the exception of bacteria and boron (about 250 samples), and only infrequent samples were analyzed for fluorescence. Coprostanol was found in about 25% of the water samples (and in about 75% of the 350 sediment samples analyzed). Caffeine was found in very few samples, while elevated *E. coli* and enterococci (using IDEXX tests) were observed in about 10% of the samples. Strong sewage odors in water and sediment samples were also detected in about 10% of the samples. Detergents and fluoride (at >0.3 mg/L) were found in about 40% of the samples and are expected to have been contaminated by industrial activities (lubricants and cleansers) and not sewerage. Overall, about 10% of the samples were therefore expected to have been contaminated with sanitary sewage, about the same rate previously estimated for stormwater systems.

Additional related laboratory tests, funded by the University of New Orleans and the EPA (Barbé et al. 2000), were conducted using many sewage and laundry detergent samples, and it was found that the boron test was a poor indicator of sewage, possibly due to changes in formulations in modern laundry detergents. Laboratory tests did find that fluorescence was an excellent indicator of sewage, especially when using specialized "detergent whitener" filter sets, but this was not very repeatable. Researchers also examined several UV absorbance wavelengths as sewage indicators and found excellent correlations with 228 nm, a wavelength having very little background absorbance in local spring waters, but with a strong response factor with increasing strengths of sewage.

Table 6.39 summarizes the different measurement parameters discussed above. We recommend that our originally developed and tested protocol (including measurement of obvious indicators, detergents, fluoride, ammonia, and potassium) still be used as the most efficient routine indicator of sewage contamination of stormwater drainage systems, with the possible addition of specific *E. coli* and enterococci measurements and UV absorbance at 228 nm. The numerous exotic tests requiring specialized instrumentation and expertise do not appear to warrant their expense and long analytical turn-around times, except in specialized research situations, or when special confirmation is economically justified (such as when examining sewer replacement or major repair options).

Hydrocarbon Fingerprinting for Investigating Sources of Hydrocarbons

Fingerprinting to identify the likely source of hydrocarbon contamination is a unique process that recognizes degradation of the material by examining a wide variety of parameters, usually by sophisticated chromatography methods. There are numerous experts who have developed and refined the necessary techniques. The following is a list of some of these expert groups, from recommendations from the Internet environmental engineering list serve group, enveng-L:

- Friedman & Bruya, Seattle, WA
- Arthur D. Little, Inc., Cambridge, MA
- GW/S Environmental Consulting, Tulsa, OK
- Public & Environmental Affairs, Indiana University, Bloomington, IN
- Graduate School of Oceanography, University of Rhode Island, Narragansett, RI
- Louisiana State University, Baton Rouge, LA
- Geological and Environmental Research Group, Texas A&M University, College Station, TX
- Trillium, Inc., Coatesville, PA
- McLaren/Hart, Inc., Albany, NY
- Phoenix Laboratories, Chicago, IL
- Golder Assoc., Mississauga, Ontario, Canada
- Daniel B. Stephens & Assoc., Albuquerque, NM
- Global Geochemistry Corp., Canoga Park, CA
- Fluor Daniel GTI, Kent, WA
- Battelle, Inc., Duxbury, MA

In addition, the University of Wisconsin, Madison, Department of Engineering Professional Development (608-262-1299) periodically offers extension classes specifically on hydrocarbon pattern recognition and dating, led by experts in the field. The IBC Group (Southborough, MA, 508-481-6400) also offers an executive forum on environmental forensics, also led by many of the above experts, that addresses many issues pertaining to the legal implications of hydrocarbon tracing.

Stout et al. (1998) prepared an overview of environmental forensics, describing how systematic investigation of a contaminated site or an event can make it possible to determine the true origin and nature of complex chemical conditions. Chemical fingerprinting, generally using high-resolution gas

Table 6.39 Comparison of Measurement Parameters Used for Identifying Inappropriate Discharges into Storm Drainage

Parameter Group	Comments	Recommendation
Fecal coliform bacteria and/or use of fecal coliform to fecal streptococci ratio	Commonly used to indicate presence of sanitary sewage.	Not very useful as many other sources of fecal coliforms are present, and ratio not accurate for old or mixed wastes.
Physical observations (odor, color, turbidity, floatables, deposits, stains, vegetation changes, damage to outfalls)	Commonly used to indicate presence of sanitary and industrial wastewater.	Recommended due to easy public understanding and easy to evaluate, but only indicative of gross contamination, with excessive false negatives (and some false positives). Use in conjunction with chemical tracers for greater sensitivity and accuracy.
Detergents presence (anionic surfactant extractions)	Used to indicate presence of wash waters and sanitary sewage.	Recommended, but care needed during hazardous analyses (only for well-trained personnel). Accurate indicator of contamination during field tests.
Fluoride, ammonia and potassium measurements	Used to identify and distinguish between wash waters and sanitary sewage.	Recommended, especially in conjunction with detergent analyses. Accurate indicator of major contamination sources and their relative contributions.
TV surveys and source investigations	Used to identify specific locations of inappropriate discharges, especially in industrial areas.	Recommended after outfall surveys indicate contamination in drainage system.
Coprostanol and other fecal sterol compounds	Used to indicate presence of sanitary sewage.	Possibly useful. Expensive analysis with GC/MSD. Not specific to human wastes or recent contamination. Most useful when analyzing particulate fractions of wastewaters or sediments.
Specific detergent compounds (LAS, fabric whiteners, and perfumes)	Used to indicate presence of sanitary sewage.	Possibly useful. Expensive analyses with HPLC. A good and sensitive confirmatory method.
Fluorescence	Used to indicate presence of sanitary sewage and wash waters.	Likely useful, but expensive instrumentation. Rapid and easy analysis. Very sensitive.
Boron	Used to indicate presence of sanitary sewage and wash waters.	Not very useful. Easy and inexpensive analysis, but recent laundry formulations in U.S. have minimal boron components.
Pharmaceuticals (colfibric acid, aspirin, ibuprofen, steroids, illegal drugs, etc.)	Used to indicate presence of sanitary sewage.	Possibly useful. Expensive analyses with HPLC. A good and sensitive confirmatory method.
Caffeine	Used to indicate presence of sanitary sewage.	Not very useful. Expensive analyses with GC/MSD. Numerous false negatives, as typical analytical methods not suitably sensitive.
DNA profiling of microorganisms	Used to identify sources of microorganisms	Likely useful, but currently requires extensive background information on likely sources in drainage. Could be very useful if method can be simplified, but with less specific results.
UV absorbance at 228 nm	Used to identify presence of sanitary sewage.	Possibly useful, if UV spectrophotometer available. Simple and direct analyses. Sensitive to varying levels of sanitary sewage, but may not be useful with dilute solutions. Further testing needed to investigate sensitivity in field trials.
Stable isotopes of oxygen	Used to identify major sources of water.	May be useful in area having distant domestic water sources and distant groundwater recharge areas. Expensive and time consuming procedure. Cannot distinguish between wastewaters if all have common source.
E. coli and enterococci bacteria	More specific indicators of sanitary sewage than coliform tests.	Recommended in conjunction with chemical tests. Relatively inexpensive and easy analyses, especially if using the simple IDEXX methods.

chromatography coupled with mass spectroscopy, is usually supplemented with site information on soils and groundwater conditions. The presentation of masses of data is usually highly visually oriented to make complex patterns and associations easier to comprehend. In addition to GC/MS, stable isotope analyses may be conducted to identify origins of very similar materials. Historical records also need to be reviewed to understand the changes that a site has undergone over the years ("corporate archaeology"). Sanborn Fire Insurance Maps (Geography and Map Division, Library of Congress) are commonly used to identify site activities during the second half of the 19th century, for example. This type of approach can be used to identify sources of contaminated sediments in urban streams, especially in areas having historical industrial activities.

Other techniques can be used to date deposits and to indicate the extent of the weathering of petroleum (Whittaker and Pollard 1997). The weathered state of spilled (or discharged) hydrocarbons can be determined using biomarkers (pristane, phthane, hopanes, and steranes) which are quite resistant to weathering processes (biotransformations and evaporation). These are therefore relatively conservative materials and can be compared to less stable oil components to indicate the extent of weathering that has occurred, and hence the approximate time since the petroleum was deposited. Other biomarkers can also be used as unique fingerprints to identify the likely source of the oil. Hurst et al. (1996) also describe how lead isotopes ($^{206}Pb/^{207}Pb$ ratio) can be used to age spilled gasoline, based on changes in gasoline additives with time.

MICROORGANISMS IN STORMWATER AND URBAN RECEIVING WATERS

As discussed in Chapter 3, microorganisms frequently interfere with beneficial uses in urban receiving waters. The use of conventional indicator organisms may be helpful, but investigations of specific pathogens is also becoming possible with new analytical technologies. The following discussion contains some background on the development of water quality standards for indicator organisms, describes some new analytical procedures, and presents an approach that measures organism die-off *in situ*, which is important for assessing the public health risk associated with water contact in urban receiving waters.

Pathogens in stormwater and urban receiving waters are a significant concern potentially affecting human health. The use of indicator bacteria is controversial for stormwater, as is the assumed time of typical exposure of swimmers to contaminated receiving waters. However, recent epidemiological studies have shown significant health effects associated with stormwater-contaminated marine swimming areas. Protozoan pathogens, especially those associated with likely sewage-contaminated stormwater, is also a public health concern.

Human health standards for body contact recreation (and for fish and water consumption) are based on indicator organism monitoring. Monitoring for the actual pathogens, with few exceptions, requires an extended laboratory effort, is very costly, and not very accurate. Therefore, the use of indicator organisms has become established. Dufour (1984a) presents an excellent overview of the history of indicator bacterial standards and water contact recreation.

Total coliforms were initially used as indicators for monitoring outdoor bathing waters, based on a classification scheme presented by W.J. Scott in 1934. Total coliform bacteria, refers to a number of bacteria including *Escherichia*, *Klebsiella*, *Citrobacter*, and *Enterobacter*. They are able to grow at 35°C and ferment lactose. They are all Gram-negative asporogenous rods and have been associated with feces of warm-blooded animals. They are also present in soil.

The fecal coliform test is not specific for any one coliform type, or groups of types, but instead has an excellent positive correlation for coliform bacteria derived from the intestinal tract of warm-blooded animals (Geldreich et al. 1968). The fecal coliform test measures *Escherichia coli* as well as all other coliforms that can ferment lactose at 44.5°C and are found in warm-blooded fecal discharges. Geldreich (1976) found that the fecal coliform test represents over 96% of the coliforms derived from human feces and from 93 to 98% of those discharged in feces from other warm-blooded animals, including livestock, poultry, cats, dogs, and rodents. In many urban runoff studies, all of the fecal coliforms were *E. coli* (Quresh and Dutka 1979). *E. coli*, a member of the fecal coliform group, has been used as a better indicator of fresh fecal contamination, compared to fecal coliforms. Table 6.40 indicates the species and subspecies of the *Streptococcus* and *Enterococcus* groups of bacteria that are used as indicators of fecal contamination.

Table 6.40 Streptococcus Species Used as Indicators of Fecal Contamination

Indicator Organism	Enterococcus Group	Streptococcus Group
Group D antigen		
Streptococcus faecalis	X	X
S. faecealis subsp. *liquifaciens*	X	X
S. faecalis subsp. *zymogenes*	X	X
S. faecium	X	X
S. bovis		X
S. equinus		X
Group Q antigen		
S. avium		X

Fecal streptococci bacteria are all of the intestinal streptococci bacteria from warm-blooded animal feces (Geldreich and Kenner 1969). The types and concentrations of different bacteria biotypes vary for different animal sources. Fecal streptococci bacteria are indicators of fecal contamination. The enterococci group is a subgroup that is considered a better indication of human fecal contamination. *S. bovis* and *S. equinus* are considered related to feces from nonhuman warm-blooded animals (such as from meat processing facilities, dairy wastes, and feedlot and other agricultural runoff), indicating that enterococcus may be a better indication of human feces contamination. However, *S. faecalis* subsp. *liquifaciens* is also associated with vegetation sources, insects, and some soils.

The EPA's evaluation of the bacteriological data indicated that using the fecal coliform indicator group at the maximum geometric mean of 200 organisms per 100 mL, as recommended in *Quality Criteria for Water* would cause an estimated eight illnesses per 1000 swimmers at freshwater beaches. Additional criteria, using *E. coli* and enterococci bacteria analyses, were developed using these currently accepted illness rates. See Appendix G for specific details of these criteria. These bacteria are assumed to be more specifically related to poorly treated human sewage than the fecal coliform bacteria indicator. It should be noted that these indicators only relate to gastrointestinal illness, and not other problems associated with waters contaminated with bacterial or viral pathogens. Common swimming beach problems associated with contamination by stormwater include skin and ear infections caused by *Pseudomonas aeruginosa* and *Shigella*.

Viruses may also be important pathogens in urban runoff. Very small amounts of a virus are capable of producing infections or diseases, especially when compared to the large numbers of bacterial organisms required for infection (Berg 1965). The quantity of enteroviruses which must be ingested to produce infections is usually not known (Olivieri et al. 1977b). Viruses are usually detected at low levels in urban receiving waters and storm runoff. Researchers have stated that even though the minimum infective doses may be small, the information available indicates that stormwater virus threats to human health are small. Because of the low levels of virus necessary for infection, dilution of viruses does not significantly reduce their hazard.

States et al. (1997) examined *Cryptosporidium* and *Giardia* in river water serving as Pittsburgh's water supply. They collected monthly samples from the Allegheny and Youghiogheny Rivers for 2 years. They also sampled a small stream flowing through a dairy farm, treated sanitary sewage effluent, and CSOs. The CSO samples had much greater numbers of the protozoa than any of the other samples. No raw sewage samples were obtained, but they were assumed to be very high because of the high CSO sample values. The effluent from the sewage treatment plant was the next highest, at less than half the CSO values. The dairy farm stream was not significantly different from either of the two large rivers. The water treatment process appeared to effectively remove *Giardia*, but some *Cryptosporidium* was found in the filtered water. Settling the river water seemed to remove some of the protozoa, but the removal would not be adequate by itself. States et al. (1997) also reviewed *Giardia* and *Cryptosporidium* monitoring data. Raw drinking water supplies were shown to have highly variable levels of these protozoa, typically up to several hundred *Giardia* cysts and *Cryptosporidium* oocysts per 100 L, and were found in 5 to 50% of the samples evaluated. Conventional water treatment appeared to remove about 90% of the protozoa.

A microorganism monitoring program for stormwater-impacted urban receiving waters could therefore be very complex and expensive if all the above organisms were to be evaluated. The bacteria (especially total coliforms, fecal coliforms, *E. coli*, enterococci, and hopefully *Pseudomonas aeruginosa*) should probably all be adequately covered in a monitoring program. Total coliforms are of most interest in marine environments based on epidemiological studies conducted in Santa Monica Bay (see case study in Chapter 4). In most cases, total coliform data could be misleading because of its ubiquitous nature (see Chapter 8). Protozoa, and especially viruses, require highly specialized analytical skills and are therefore not likely to be routinely investigated. However, protozoa are being more commonly monitored, especially with new federal regulations to protect drinking water supplies.

Sampling for microorganism evaluations is more challenging than for most constituents, requiring sterile sample containers and tools, plus rapid shipment of the samples to the laboratory and immediate initiation of analyses. Bacteriological analyses are becoming much more simplified with special procedures and methods developed by HACH, Millipore, and IDEXX Corp., for example. Available methods require little more than mixing a freeze-dried "reagent" with a measured amount of sample, pouring the mixture into special incubation trays and sealing them, and finally placing them into incubators for the designated time (usually 18 to 48 hours).

The IDEXX method for *E. coli*, Colilert-18 (see Figures 6.71 through 6.74), is used by many state agencies for EPA reporting purposes. It is used for the simultaneous detection, specific identification, and confirmation of total coliforms and *E. coli* in water. It is based on IDEXX's patented Defined Substrate Technology® (DST™). It is a most probable number (MPN) method. Colilert-18 utilizes nutrient indicators that produce color and/or fluorescence when metabolized by total coliforms and *E. coli.* When the Colilert-18 reagent is added to a sample and incubated, it can detect these bacteria at 1 cfu in 100 mL within 18 hours with as many as 2 million heterotrophic bacteria per 100 mL present. The required apparatus includes the Quanti-tray sealer, an incubator, a 6-watt 365-nm UV light, and a fluorescence comparator. This procedure requires 100 mL of sample, which should be analyzed ASAP after sampling. Marine water samples must be diluted at least tenfold with sterile fresh water to reduce the salinity. Quality control includes testing with cultures of *E. coli, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.

The Enterolert procedure, also from IDEXX, is very similar to the Colilert method outlined above. Enterolert is used for the detection of enterococci such as *Enterococcus faecium* or *E. faecalis* in fresh and marine water. When the Enterolert reagent is added to a sample and incubated, bacteria down to 1 CFU in a 100 mL sample can be detected within 24 hours. This method also has a quality control procedure that should be conducted on each lot of Enterolert, using test cultures of *E. faecium*, *Serratia marcescens* (Gram-negative), and *Aerococcus viridans* (Gram-positive).

Determination of Survival Rates for Selected Bacterial and Protozoan Pathogens

The following discussion was prepared by John Easton while he was a Ph.D. student at the University of Alabama at Birmingham and describes some of the experiments he has conducted concerning the survival of wet-weather flow bacteria and pathogens after being discharged to urban receiving waters (Easton 2000). This section is not intended to be a comprehensive review of survival of microorganisms in the environment, but is intended to illustrate how actual site-specific survival rates can be determined, especially for unusual conditions (affected by water temperature, turbidity, natural predation, local sources and receptors, etc.). This information is necessary for human health assessments when predicting resulting downstream pathogen conditions. Much of the literature on microorganism survival is based on laboratory investigations that might not be applicable to actual field conditions. The simple tests described in this section allow more accurate in-stream predictions.

Pathogenic organisms found in sewage can adversely impact public health when the sewage is discharged to waters that humans come in contact with when wading, swimming, fishing, drinking, etc. UAB is conducting research to develop a risk assessment methodology for evaluating varying degrees of risk related to human contact with pathogenic microorganisms found in sewage-contaminated waters, especially those caused by separate sanitary sewer overflows (SSOs). One component of this research is to study the fate and transport of these microorganisms in the environment. The survivability, or die-off, rates for these organisms are critical to understanding their fate in the environment, e.g., from an SSO discharge through a receiving water.

Microorganisms have varying degrees of stability within the environment. Their numbers are dependent upon population dynamics, which is controlled by several criteria (McKinney 1992): (1) competition for food (limited food sources limit microbial numbers), (2) predator–prey relationships (some organisms consume others for food sources), (3) nature of organic matter (carbohydrates, organic acids, and proteins all stimulate different organisms), and (4) environmental conditions (oxygen concentration, nutrient levels, temperature, pH, etc.). Since there are a multitude of factors that contribute to microorganism survivability, the use of an *in situ* method to characterize the rates of growth and death is necessary to account for variable environmental conditions.

Several experiments were conducted to evaluate the rate of die-off, or decay, for the study microorganisms. These *in situ* experiments were conducted in specially designed chambers (Figure 6.118). These were designed to allow passage of water and nutrients between the inside of the chamber and the outside environment (Five-Mile Creek in Jefferson County, AL), while sequestering the microorganisms inside to allow enumeration at various times during the experiment.

These experiments included exposures over a 21-day period. A polyethersulfone (Supor®, Gelman Sciences) membrane filter, which is not susceptible to biological degradation, was used. This membrane material was clamped onto either end of a piece of acrylic tubing in a design devised by researchers at UAB (Figure 6.119). The membrane pore size is 0.22 μm, allowing exchange of ions with the surrounding water while sequestering the microorganisms inside the test chamber.

Multiple chambers containing sewage samples were placed in the creek and removed after 0, 1, 3, 7, 10, 14, and 21 days. For each time point, three separate chambers were removed and composited for analysis. Once the samples were composited, they were blended (Waring blender for 2 min) to minimize agglomeration of the microorganisms.

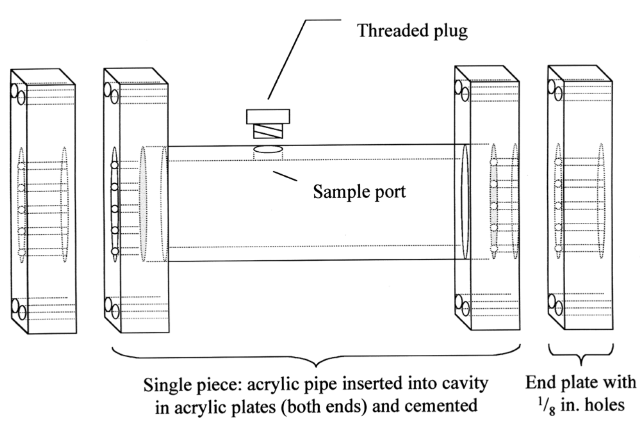

Figure 6.118 Acrylic components of *in situ* chamber. (From Easton, J. *The Development of Pathogen Fate and Transport Parameters for Use in Assessing Health Risks Associated with Sewage Contamination.* Ph.D. dissertation, the Department of Civil and Environmental Engineering, University of Alabama at Birmingham. 2000. With permission.)

The experiments conducted to evaluate degradation of *G. lamblia* were conducted *in situ*. The sewage matrix was spiked with approximately 10,000 cysts per liter to enable detection after significant die-off. These cysts were formalinized in order not to risk releasing a potentially infectious pathogen into the environment. Since these organisms are in cyst form, i.e., relatively inert, it was hypothesized that the mechanism of die-off would be predation by other organisms and formalinized organisms would be a suitable surrogate for "live" ones.

The results of these experiments show that the microorganisms die off at a constant, rapid rate (assumed in most receiving models) only for an initial short period. As time progressed, the die-off rate slows. Figure 6.120 is a plot of the levels of *Giardia* cysts vs. time. The method used to enumerate these organisms (EPA method 1623) requires a presumptive test followed by a confirmed test. The presumptive test consists of identifying objects of the correct size and shape which are stained by a *Giardia*-specific antibody bound to a fluorescent probe. Next, the organisms are confirmed by identification of internal structures stained by the nuclear stain DAPI (4′,6-diamindino-2-phenylindole). Unfortunately, problems were encountered with the confirmation test in these

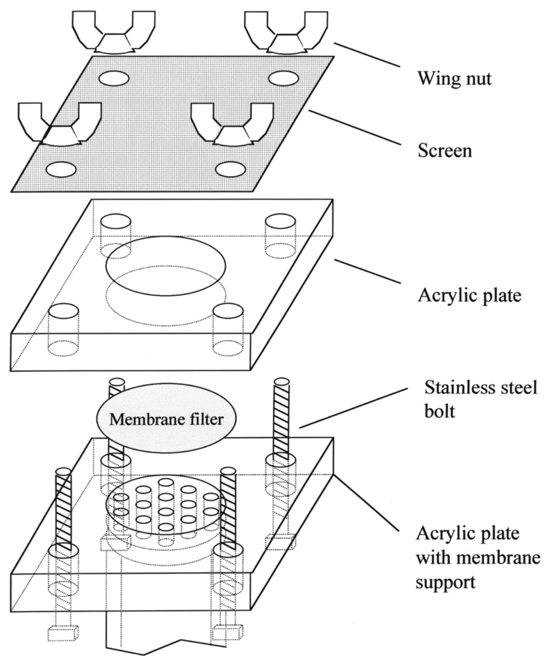

Figure 6.119 End-plate of *in situ* chamber showing the location of membrane filter. (From Easton, J.H. et al. The use of a multi-parameter water quality monitoring instrument to continuously monitor and evaluate runoff events. Presented at *Annual Water Resources Conference of the AWRA,* Point Clear, AL. 1998. With permission.)

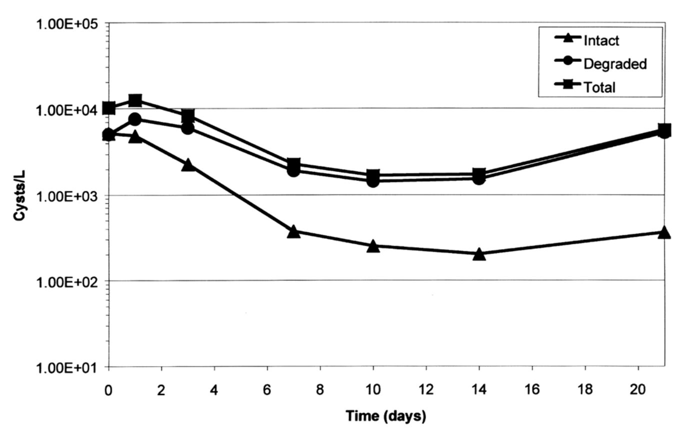

Figure 6.120 Degradation plot of *Giardia* cysts. (From Easton, J.H. et al. The use of a multi-parameter water quality monitoring instrument to continuously monitor and evaluate runoff events. Presented at *Annual Water Resources Conference of the AWRA,* Point Clear, AL. 1998. With permission.)

experiments (the DAPI stain of the background was too intense to enable identification of internal structures). However, using the presumptive stain, which binds to the cyst cell wall, it was possible to detect differences in these presumptive *Giardia* cysts. Some cysts were intact (i.e., the stain covered the cell wall continuously), and some cysts were present but degraded (i.e., the staining of the cell wall was less intense and not continuous). The levels of the former, "intact cysts," are plotted along with the levels of the latter, "degraded cysts," in Figure 6.120.

Since the microorganisms' rate of die-off seems to decrease over time, the regression model was applied stepwise, starting with the first three data points and adding one additional point until the entire 21-day, or 7-point, data set was used. In general, the die-off rates decreased, and T_x values correspondingly increased as data over longer time periods are included in the regression analyses. The T_{90} values (time needed for 90% die-off) for the indicator bacteria, total coliforms and *E. coli*, are in accordance with conventional wisdom. Many studies have shown T_{90} values for these organisms to be in the range of several hours to a few days (Droste and Gupgupoglu 1982; Geldreich et al. 1968; Geldreich and Kenner 1969). The initial, rapid die-off occurred, generally, within the first 7 days of the experiment. Table 6.41 gives a first-order die-off constant, k (days^{-1}), and its associated 95% confidence interval, for each of the microorganisms. In addition, the results of the Mann–Kendall Test (a nonparametric test for trend) are given. All of the die-off constants (slope of the regression line) are statistically significant except for enterococci.

Table 6.41 Die-off Rates Determined Using Day 0 to Day 7 Data

Organism	Die-off Rate (day^{-1})	95% CI	Mann–Kendall Trend[a]
Total coliforms	–0.310	± 0.152	$p = 0.042$
E. coli	–0.331	± 0.049	$p = 0.042$
Enterococci	–0.078	± 0.189	$p = 0.375$[b]
Giardia	–0.171	± 0.074	$p = 0.042$

[a] $p < 0.05$ indicates significant downward trend.
[b] Not significant, no trend (die-off).

From Easton, J. *The Development of Pathogen Fate and Transport Parameters for Use in Assessing Health Risks Associated with Sewage Contamination.* Ph.D. dissertation, the Dept. of Civil and Environmental Engineering, University of Alabama at Birmingham. 2000. With permission.

The data generated by this study suggest that if one were using die-off constants from indicator bacteria studies, then one may tend to underpredict the length of time or distance downstream in which adverse health effects due to pathogens in sewage are present. In addition, these data indicate that assumptions regarding the constancy of die-off rates may be invalid. There seems to be a modulation of the rate of die-off with increased time, as all of the test organisms showed a pattern of leveling off toward some equilibrium level with increasing time.

The *Enterococcus* results are quite different from the others, with no rapid initial die-off, as generally reported in the literature (Facklam and Sahm 1995). This persistence is due to the enterococci being Gram-positive and is therefore a better indicator of virus survival. For these reasons, the EPA has selected enterococci as an indicator organism in their new guidance documents.

The *Giardia* results were not as expected. The descriptions of this organism found in the literature seem to predict that *Giardia* will persist much longer than observed in these tests. This study seems to show that *Giardia*, and perhaps other protozoan pathogens, exhibits die-off characteristics similar to the bacteria included in this study. However, these cysts were treated with formalin and therefore may have been less resistant to degradation in the environment.

There are many stormwater microorganisms of interest when conducting a receiving water study. However, besides characterizing microorganism conditions, it is also necessary to understand population dynamics when predicting fate and exposures. This section briefly described some of the currently used analytical methodologies for measuring microorganism counts, along with an example *in situ* die-off experiment.

BENTHOS SAMPLING AND EVALUATION IN URBAN STREAMS

Ecosystem degradation via water, sediment, and habitat alteration affects food resources, reproduction, growth, and survival of aquatic biota, thereby altering the structure and functioning of the system. Structural indicators include the number and kinds of individuals, species, population, and communities as measured by a variety of metrics. The structural alterations may impact ecosystem functions such as productivity, respiration, organic matter degradation, nutrient cycling, and energy flow, which, unfortunately, are often difficult to quantify and are resource-demanding. A useful way to measure functional changes is an indirect method whereby organisms are placed into trophic categories (e.g., predators/consumers, producers, omnivores, detritivores), which allows production and consumption dynamics to be measured. This concept has been described by Cummins (1974, 1975) and Vannote et al. (1980) in stream ecosystems as a predictable and continuous gradient of interrelated physical, structural, and functional characteristics (Table 6.42). When conditions deviate from those in reference streams of a similar stream order for that ecoregion, then impacts may be occurring.

Bottom-dwelling organisms comprise all the major trophic levels including decomposers, photosynthetic organisms (algae and macrophytes), and herbivorous and carnivorous animals. These communities live on or in the sediment or other solid surfaces (e.g., roots, decaying wood, rocks) for significant parts of their life cycle. The fauna and flora studied in environmental quality assessments have ranged from small to large, using bacteria, phytoplankton, macrophytes, protozoa, worms, crustaceans, molluscs, insects, and fish (Burton 1991). Fish will be discussed in a following subsection. The major component of benthic fauna is often the bacteria, segmented worms (e.g., oligochaetes), microcrustacea (e.g., ostracods), macrocrustacea (e.g., isopods, decapods, amphipods), and immature insects (e.g., chironomids, plecoptera, trichoptera, and ephemeroptera). Of these major groups, the immature insects have received the greatest amount of study. Consequently, there is a large database concerning life history information and relative pollution sensitivity. The major aquatic insect groups are Odonata (dragonflies), Ephemeroptera (mayflies), Plecoptera (stoneflies), Trichoptera (caddisflies), Coleoptera (beetles), and Diptera (flies, midges, mosquitoes). Each group varies in its pollution sensitivity. Each goes through multiple life stages and molts, often emerging from the water as adults. Life cycles range from a few weeks to 2 years. See Pennak (1989) and Merritt and Cummins (1984) for more information on life histories. The sedentary (nontransitory) nature of most benthic species makes them ideal chronic, long-term pollution indicators, as compared to migratory fish or other species, such as zooplankton.

The micro-, meiofauna and flora may play a major role in the aquatic ecosystem's functioning, such as photosynthetic production by periphyton, and organic matter and nutrient processing–cycling by a variety of microbial populations and communities. These groups have temporal spatial dynamics and microhabitat requirements that are much different from the macrofauna and flora, and in many respects are more difficult to study (Burton 1991). For holistic, integrative ecosystem assessments of stormwater impacts, it is necessary to define effects on the benthic microbial communities, which will require additional expertise and resources. Further information is available from Burton et al. (2000) and the annual review issue of *Water Environment Research*. Most studies, however, whose objectives are to assess stormwater effects on receiving waters, will focus on the macroinvertebrate component of the benthos. This is not because they are more important than the meio- or microbenthos, but rather because they are more effectively used in pollution assessments. The following discussions highlight some of the important benthic groups and the characteristics one should consider in their sampling and evaluation.

Table 6.42 General Characteristics of Running Water Ecosystems According to Size of Stream

Stream Size	Primary Energy Source	Production (trophic) State	Light and Temperature Regime	Trophic Status of Dominant Insects	Fish
*Small headwater streams (stream order 1–3)	Coarse particular organic matter (CPOM) from the terrestrial environmental	Heterotrophic	Heavily shaded	Shredders	Invertivores
	Little primary production	P/R < 1	Stable temperatures	Collectors	
*Medium-sized streams (4–6)	Fine particulate organic matter (FPOM),mostly	Autotrophic	Little shading	Collectors	Invertivores
	Considerable primary production	P/R < 1	High daily temperature variation	Scrapers (grazers)	Piscivores
Large rivers (7–12)	FPOM from upstream	Heterotrophic	Little shading	Planktonic	Planktivores
		P/R < 1	Stable temperatures	Collectors	

* Streams are typically subdivided into three size classes based on the stream order classification system of Kuehne (1962).

Modified from Cummins, K.W. Ecology of running waters: theory and practice, in *Proc. Sandusky River Basin Symposium.* Edited by D.B. Baker. Heidelburg College, Tiffin, OH. 1975.

Periphyton Sampling

Periphyton is a general descriptor which can encompass epipelic (sediment surface), epilithic (stone surface), and epiphytic (plant surface) algae and other benthic meio-, microorganisms. Most periphyton studies have focused on the diatom group, which frequently dominates. Green algae, blue-green algae (cyanobacteria), and flagellates are also dominant species in some sediments, with diatoms favoring calcareous sediments (Wetzel 1975). The animal communities which may be present include protozoa, rotifers, nematodes, and bryozoans. A major controlling factor is light. In turbid, eutrophic, shaded, or deep waters, the low light levels may restrict photosynthetic activity (Wetzel 1975). Some epipelic algae appear to have a diurnal migration pattern through the top few centimeters of sediment in response to light availability. Their photosynthetic activity causes a diurnal change in oxygen concentrations with the upper few millimeters of sediment (Carlton and Klug 1990), which may affect metal bioavailability. They serve as an important transformation link for nitrogen, assimilating pore water ammonia and excreting organic nitrogen to overlying waters, and may be the primary productivity source (Wetzel 1975). These communities have less temporal fluctuation in a lake than planktonic algae and may have one to two biomass peaks per year (Wetzel 1975). Some algae present on sediment surfaces may have settled from the water column and can resuspend to overlying waters.

The algal community is not only extremely important in aquatic ecosystems, but has several attributes as a monitoring tool. Algae have short life cycles. Therefore, they indicate recent-to-present water quality conditions. They are directly affected by physical and chemical conditions since they are primary producers. Sampling of indigenous algae is nondestructive, easy, and inexpensive, and traditional assessment methods exist. Finally, they represent a unique level of biological organization and are sensitive to contaminants which may not be detected with nonalgal surrogates.

Periphyton is difficult to study in a quantitative manner when collecting from natural substrates, as small particle size-surface area differences between samples or sites can have significant effects. Often-used taxonomic references for algae and diatoms include Smith (1950), Prescott (1962, 1970), and Patrick and Reimer (1966). The use of artificial substrates for periphyton and other benthic communities removes the substrate variable. Natural substrates may be sampled using the methods of Stevenson and Lowe (1986) or Hamala et al. (1981). A commonly used artificial sampler (diatometer) consists of multiple glass slides suspended from a floating holding frame (APHA 1985; Figure 6.121; also see Figure 4.11 illustrating the use of a diatometer in Coyote Creek, San Jose, CA). Not all species will colonize the glass slides, but the advantages of / efficient and precise evaluations outweigh this / disadvantage in most cases. Valid station comparisons are only possible when the key variables / affecting periphyton communities are similar; these include flow, turbidity, temperature, dissolved / oxygen, alkalinity, hardness, conductivity, nutrients (APHA 1985), and photosynthetically active/ radiation (LiCor 1979). /

Figure 6.121 Diatometer for artificial substrate pe phyton sampling.

The periphyton community can be evaluated for stormwater effects using several endpoints. When using a diatometer, slides should be left *in situ* for 6 to 14 days, then placed in formalin upon collection. Evaluation endpoints may include: number, richness, relative abundance, diversity, chlorophyll *a*, and other community or productivity indices (APHA 1985; Crossey and LaPoint

1988). Stevenson and Lowe (1986) recommend counting 200 cells for dominant, 500 for uncommon, and 1000 cells for rare species, or an additional 100 cells for each new species encountered (EPA 1989a,b,c, 1999).

Periphyton community analyses may be of a structural and functional nature. Structural measures include diversity indices, taxa richness, indicator species, and biomass (Rodgers et al. 1979; Wetzel 1979; Palmer 1977; Patrick 1973). Functional measures which have been used are primary productivity (e.g., chlorophyll *a*), or respiration (Rodgers 1979). Integrating the structural and functional characteristic provides the best means of evaluating ecosystem health, as demonstrated in the macroinvertebrate and fish approaches below.

Protozoan Sampling

Protozoans, like algae, exist in the planktonic and benthic communities. Because their biomass is relatively low compared to that of other aquatic communities, their contribution as a food source to higher trophic levels is probably limited; however, their function as predators or decomposers may fill important ecosystem niches and assist in maintaining–stabilizing decomposition and cycling processes. When protozoan cropping of bacteria is removed, the sediments can function as a carbon sink and microbial community structure–function relationships could alter, affecting nutrient availability to higher trophic levels (Griffiths 1983; Porter et al. 1987).

Several studies have shown the effective use of artificial (polyurethane) substrates in water and sediment pollution studies (Pontasch et al. 1989; Henebry and Ross 1989). This approach allows the foam substrates to colonize at reference sites for several days. Then they are exposed to toxicants either in the laboratory or *in situ* to test sample waters and compared to reference responses. The test endpoints of this multispecies assay include decolonization, protozoan abundance, taxa number, phototroph and heterotroph abundance, respiration, and island-epicenter colonization rates. Both stimulatory and inhibitory results are observed, and careful interpretation is required (Henebry and Ross 1989).

Macroinvertebrate Sampling

This group is operationally defined as those invertebrates retained on sieve mesh sizes greater than 0.2 mm (Hynes, 1970); however, the larger size of 0.5 or 0.95 mm (U.S. Standard No. 30) is used routinely (EPA 1989c). More representative benthos samples may be collected using smaller mesh sizes, such as 0.25 mm (U.S. Standard No. 60), which collect early life stages, chironomids, and nadid and tubificid oligochaetes (EPA 1990b). The major freshwater taxonomic groups may be separated into the trophic levels — functional feeding group descriptors of herbivores, omnivores, carnivores; or deposit and detritus feeders, collectors, shredders, grazers; or scrapers, parasites, scavengers, and predators (EPA 1990b). In most studies of high-to-medium-quality waters, species level identification will be necessary, with tolerant species only dominating in polluted systems. Each taxonomic group may contain a variety of functional feeding groups (Table 6.43). Some common pollution indicators are shown in Figure 6.122.

The benthic macroinvertebrate community has been used for many years to qualitatively and, more recently, to quantitatively assess water quality and pollution effects. There are advantages and disadvantages in using macrobenthos in water quality assessments (Table 6.44). However, except in cases of extreme and obvious pollution, they should always be a component of a stormwater effect assessment.

There is a wealth of reference information available to assist in the use of macroinvertebrates as monitoring tools, including Armitage (1978), Benke et al. (1984), Brinkhurst (1974), Cairns (1979), Cummins et al. (1984), Cummins and Wilzbach (1985), Edmondson and Winberg (1971), Goodnight and Whitley (1960), Hart and Fuller (1974), Hellawell (1978, 1986), Hilsenhoff (1977), Howmiller and Scott (1977), Hynes (1960, 1970), Holme and McIntyre (1971), Hulings and Gray (1971),

Table 6.43 Trophic Mechanisms and Food Types of Aquatic Insects

General Category Based on Feeding Mechanism	General Particle Size Range of Food (μm)	Subdivision Based on Feeding Mechanisms	Subdivision Based on Dominant Food	Aquatic Insect Taxa Containing Predominant Examples
Shredders	>103	Chewers and miners	Herbivores: living vascular plant tissue	Trichoptera (*Phryganeidae, Leptoceridae*) Lepidoptera Coleoptera (*Chrysomelidae*) Diptera (*Tipulidae, Chironomidae*)
		Chewer and miners	Detritivores (large particle detritivores): decomposing vascular plant tissue	Plecoptera (*Filipalpia*) Trichoptera (*Limnephilidae, Lepidostomatidae*) Diptera (*Tipulidae, Chironomidae*)
Collectors	<103	Filter or suspension feeders	Herbivores-detritivores: living algal cells, decomposing vascular plant tissue	Ephemeroptera (*Siphionuridae*) Trichoptera (*Philopotamidae, Psychomyidae, Hydropsychidae, Brachycentridae*) Lepidoptera Diptera (*Simuliidae, Chironomidae, Culicidae*)
		Sediment or deposit (surface) feeders	Detritivores (fine particle detritivores): decomposing organic particulate matter	Ephemeroptera (*Caenidae, Ephemendae, Leptophlebiidae, Baetidae, Ephemerellidae, Heptageniidae*) Hemiptera (*Gerridae*) Coleoptera (*Hydrophilidae*) Diptera (*Chironomidae, Ceratopogonidae*)
Scrapers	<103	Mineral scrapers	Herbivores: algae and associated microflora attached to living and nonliving substrates	Ephemeroptera (*Heptageniidae, Baetidae, Ephemerellidae*) Triochoptera (*Glossosomatidae, Helicopsychidae, Molannidae, Odontoceridae, Goreridae*) Lepidoptera Coleoptera (*Elmidae, Psephenidae*) Diptera (*Chironomidae, Tabanidae*)
		Organic scrapers	Herbivores: algae and associated attached microflora	Ephemeroptera (*Caenidae, Leptophlebiidae, Heptageniidae, Baetidae*) Hemiptera (*Corixidae*) Trichoptera (*Leptoceidae*) Diptera (*Chironomidae*)
Predators	>103	Swallowers	Carnivores: whole animals (or parts)	Odonata Plecoptera (*Setipalpia*) Megaloptera Trichoptera (*Rhyacophilidae, Polycentropidae, Hydropsychidae*) Coleoptera (*Dytiscidae, Gyrinnidae*) Diptera (*Chironomidae*)
		Piercers	Carnivores: cell and tissue fluids	Hemiptera (*Belastomatidae, Nepidae, Notonectidae, Naucoridae*) Diptera (*Chironomidae*)

Lenat (1983), Lind (1985), Merritt and Cummins (1984), Mason (1981), Metcalfe (1989), Milbrink (1983), Meyer (1990), Neuswanger et al. (1982), Pennak (1989), Posey (1990), Resh (1979), Resh and Roseberg (1984), Resh and Unzicker (1975), Reynoldson et al. (1989), Ward and Stanford (1979), Warren (1971), Waters (1977), Welch (1948), Welch (1980), Winner et al. (1975), EPA (1989a,c, 1990a,c, 1999), and OEPA (1989). Previous discussions highlighted the importance of attempting to control habitat (e.g., substrate), flow dynamics, and seasonal variables when monitoring — particularly the benthic macroinvertebrate community. Other, obviously critical issues, include the sampling procedure's precision and accuracy, taxonomic identification, and data evaluation.

Substrates can be sampled with nets, grab (dredge), core, and vegetation collection devices (Table 6.45). The Hess and Surber samples are often used to sample stream riffle habitats, whereby the substrates within a confined 0.1 m^2 area are vigorously disrupted and scrubbed down to a depth of approximately 10 cm. A flow velocity of at least 0.5 m/s is required for effective use of these net samplers. See also ASTM (1987) for additional information.

Sampling is frequently of a qualitative to semiquantitative nature that is relatively easy to conduct. The objective here is to determine differences between sites. Semiquantitative methods incorporate a level-of-effort constant or use quantitative methods in a nonrandom manner (EPA 1990b). Quantitative methods sample unit areas or volumes of habitat in a random manner. The approach chosen should depend on the data quality objectives (DQOs).

Semi- and quantitative sampling may use grab samplers (see Chapter 5 and Table 6-45), stream net samplers (Figure 6.123 and Table 6.46), and artificial substrates (Figures 6.124 through 6.127 and Table 6.47).

In large streams, deep waters, and areas of slow current velocities, it is necessary to use core or dredge samplers, which are also used for sediment sampling as discussed previously (Chapter 5). See also ASTM (1987, 1991), Lind (1979), APHA (1985), Downing (1984), and Wetzel and Likens (1991), for additional sampler information. The Ekman and Ponar grab samplers are commonly used in relatively soft sediments of clay to gravel size, with relatively good efficiency (Elliott and Drake 1981). The hand and gravity corers are preferred in soft sediments because pressure waves and loss of surficial fines are reduced, variance can be determined horizontally and vertically, sieving volume is reduced, precision is increased, and sediment structure-integrity is maintained to

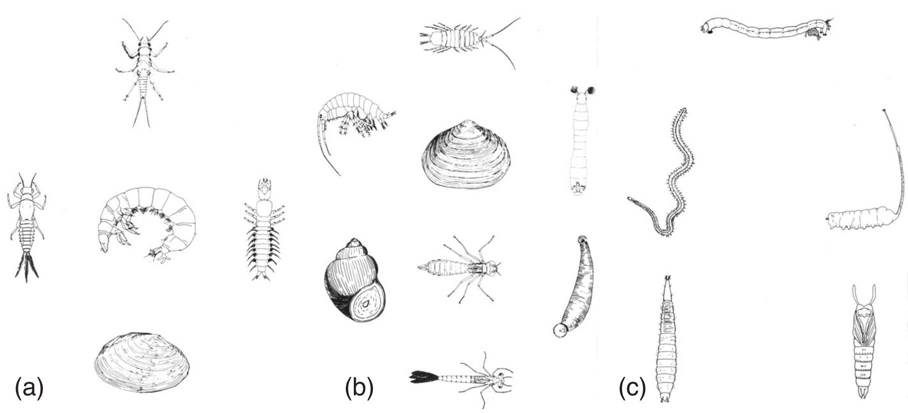

Figure 6.122 Representatives of stream bed animals commonly associated with various degrees of organic pollution. (a) The clean water (sensitive) group (from left): stonefly nymph, mayfly naiad, caddisfly larvae, hellgrammite, unionid clam. (b) The intermediately tolerant group (from left): scud, sowbug, blackfly larvae, fingernail clam, snail, dragonfly nymph, leech, damselfly nymph. (c) The very tolerant group (from left): bloodworm or midge larvae, sludgeworm, rattailed maggot, sewage fly larvae, sewage fly pupae. (From *The Practice of Water Pollution Biology.* U.S. Department of the Interior. Washington, D.C. 1969.)

Table 6.44 Advantages and Disadvantages of Using Macroinvertebrates and Fish in Evaluation of the Biotic Integrity of Freshwater Aquatic Communities

Advantages	Disadvantages
Macroinvertebrates	
Fish, highly valued by humans, are dependent on benthic invertebrates as a food source. Many species are sensitive to pollution Bottom fauna often have a complex life cycle of a year or more, and, therefore, represent long-term exposure periods to water and sediment conditions. Many have an attached or sessile mode of life and are not subject to rapid migrations, therefore serve as resident monitors of test site quality.	They require taxonomic expertise for identification, which is also time-consuming. Background life-history information is lacking for some species and groups. Results are difficult to translate into values meaningful to the general public. May not detect short-term or recent chronic pollution events. Not as sensitive a pollution indicator in large rivers, bays, lakes, and marine systems. Natural levels of spatial and temporal variation may make detection of significant effects difficult.
Fish	
Life history information is extensive for most species. Fish communities generally include a range of species that represent a variety of trophic levels (omnivores, herbivores, insectivores, planktivores, piscivores) and utilize foods of both aquatic and terrestrial origin. Their position at the top of the aquatic food web also helps provide an integrated view of the watershed environment. Fish are highly valued by the public. Fish are relatively easy to identify. Most samples can be sorted and identified in the field, and then released. Both lethality and stress effects (depressed growth, lesions, abnormalities, and reproductive success) can be evaluated. Careful examination of recruitment and growth dynamics among ages of fish can help pinpoint periods of unusual stress.	Sampling fish communities is selective in nature. Fish are highly mobile. This can cause sampling difficulties and also creates situations of preference and avoidance. Fish also undergo movements on diurnal and seasonal time scales. This increases spatial and temporal variability, which makes detection of significant effects or trends difficult. There is a high requirement for manpower and equipment for field sampling.

Modified from Cairns, J., Jr. and K.L. Dickson. A simple method for the biological assessment of the effects of waste discharges on aquatic bottom-dwelling organisms. *J. Water Pollut. Control Fed.,* 43: 755–772. 1971; Karr, J.R. and D.R. Dudley. Ecological perspective on water quality goals. Ecological perspective on water quality goals. *Environ. Manage.*, 5: 55–68. 1981. With permission.

Table 6.45 Sampling Methods for Macroinvertebrates

Method	Habitat	Substrate Type	Effort Required[a] Persons	Effort Required[a] Time (hr)	Ref.
Hess, Surber	Stream riffle (<0.5 m deep)	Sand, gravel, cobble	1	0.50	ASTM (1987)
Ponar grab	Rivers, lakes, estuaries	Mud, silt, sand, fine gravels	2	0.50	ASTM (1987)
Ekman grab	Stream pools, shallow lakes	Mud, silt, sand	1	0.25	ASTM (1987)
Corers	Rivers, lakes	Mud, silts	1–2	0.25	Downing (1984)
Sweep net	Littoral	Vegetation	1	0.25	Downing (1984)
Macan McCauley Minto Wilding	Littoral	Vegetation	1	0.50	Downing (1984)
Standardized substrates	All	All	1	0.25–1.0[b]	APHA (1985)

[a] Effort includes time spent in field to collect, sieve, and isolate one sample. Laboratory time required to remove and identify organisms ranges from 1 to 5 per sample, depending on expertise level, and taxonomic resolution sought.
[b] Two- to six-week colonization time ended before sample is removed.

Modified from EPA. *Ecological Assessment of Hazardous Waste Sites.* Environmental Research Laboratory. U.S. Environmental Protection Agency, Corvallis, OR. EPA 600/3-89/013. 1989a.

a much higher degree than in dredge samples. The principal disadvantages, however, are their ineffective sampling of coarse, large-grained sediments and the small volumes that are collected.

The efficiency of benthic collection samplers has been compared, and, in general, the grab samplers are less efficient than the corers (ASTM 1991a). The Ekman dredge is the most commonly used sampler for benthic investigations (Downing 1984). The Ekman is limited to less compacted, fine-grained sediments, as are the corer samplers. However, these are usually the sediments of greatest concern in toxicity assessments. The most commonly used corer is the Kajak–Brinkhurst, or hand corer. In more resistant sediments, the Petersen, Ponar, Van Veen, and Smith–McIntyre dredges are used most often (Downing 1984). Based on studies of benthic macroinvertebrate populations, the sediment corers are the most accurate samplers, followed by the Ekman dredge, in most cases (Downing 1984). For consolidated sediments, the Ponar dredge was identified as the most accurate, while the Petersen was the least effective (Downing 1984).

Quantitative benthic macroinvertebrate sampling of small streams may be improved by also using small to large emergence traps. These samplers trap the dominant stream insects as they leave the water as flying adults. In this way, effects from habitat heterogeneity are reduced, time-consuming “bug” picking from substrate samples is avoided, and most adult stages can be identified to the species level. See also Wetzel and Likens (1991), Illies (1971), Hall et al. (1980), and Peckarsky (1984).

Semiquantitative methods also include the traveling kick method (Horning and Pollard 1978) and the Rapid Bioassessment Protocols II and III (kicknets) (EPA 1990b). Readers should note that the EPA Rapid Bioassessment Protocol manual has been revised (EPA 1999) and no longer differentiates Protocols I through III (EPA 1989c). As with other sediment-associated components, quantitative evaluations are complicated by often high degrees of variability. By using multimetric (indice) assessment endpoints, the impact of population variability can be reduced (EPA 1990b). Nevertheless, it is essential that replicate sampling of each habitat niche be conducted at each site, allowing measures of precision. Precision may also be increased by collecting larger samples, thus the influence of reducing small patches. Three to five replicates are a minimum requirement. Use of a transect to select replicate sites may result in different habitats being selected.

A number of artificial substrate samplers have been used to assess benthic macroinvertebrate conditions, i.e., flow, depth, light, and temperature. These samplers remove the substrate variable and provide known sampling areas and exposure times. Unfortunately, there are some disadvantages which may be significant, including some taxa may not utilize the substrate; proportional relationships may be altered; substrates are colonized primarily by upstream “drift” organisms, and effects from contact with possibly contaminated bed sediments is reduced or eliminated; they require 4- to 8-week exposures and two sampling trips; and they may be lost due to high flow or vandalism (see Figures 6.123 to 6.127). As with the periphyton samplers, care must be taken to ensure uniformity.

For most studies, semiquantitative approaches using the EPA’s Rapid Bioassessment Protocols II or III (RBP) and the Ohio EPA Hester–Dendy samplers (Figure 6.127) are preferred, with habitat evaluations. The RBP II method samples 1 m^2 riffle areas, and 100 organisms are randomly picked and identified to the family level (EPA 1989c). The Ohio EPA method uses 10 metrics (nine based on Hester–Dendy results and one based on dip net sampling) to compute an Invertebrate Community Index in wadeable streams (Ohio 1989). In streams where rocks are the dominant habitat, it may be useful to use a basket sampler (Figure 6.124) containing approximately 30 rocks of equal size or a particle size distribution similar to the test or reference site. This approach is used by the State of Maine and by other investigators (e.g., Clements et al. 1996). It is the most realistic artificial substrate method. When high quantities of biomass are needed, such as for tissue residue analyses, the grill-basket sampler containing 3M polyethylene mesh is useful (Stauffer et al. 1974). All of the artificial substrates are set out in triplicate and secured to concrete blocks in shallow waters for 4 to 8 weeks. The metrics vary in their ability to detect organic material or toxicant-related impacts. They overlap in ranges of sensitivity and thereby reinforce final conclusions regarding the condition of the system’s biological communities (EPA 1989c). The RBP II methods, organism pollution tolerance levels, and indices calculated in the RBP and Ohio EPA methods are described in detail in Appendix B. Note that in many states, special collection permits are required to collect macroinvertebrates.

Table 6.46 Comparison of Stream-Net Samplers

Type	Habitats and Substrates Sampled	Effectiveness of Device	Advantages	Limitations
Surber sampler	Shallow, flowing streams, less than 32 cm in depth with good current; rubble substrate, mud, sand, gravel	Relatively quantitative when used by experienced biologist; performance depends on current and substrate	Encloses area sampled; easily transported or constructed; samples a unit area	Difficult to set in some substrate types, that is, large rubble; cannot be used efficiently in still slow-moving streams
Portable invertebrate box sampler, Hess stream bottom sampler, and stream-bed fauna sampler	Same as Surber	Same as Surber	Same as above except completely enclosed with stable platform; can be used in weed beds	Same as Surber
Drift nets	Flowing rivers and streams; all substrate types	Relatively quantitative and effective in collecting all taxa which drift in the water column; performance depends on current velocity and sampling period	Low sampling error; less time, money, effort; collects macroinvertebrates from all substrates, usually collects more taxa	Unknown where organisms come from; terrestrial species may make up a large part of sample in summer and periods of wind and rain; does not collect nondrifting organisms

From EPA. *Macroinvertebrate Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters.* Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C., EPA 600/4-90/030. 1990.

Table 6.47 Comparison of Substrate Samplers

Type of Substrate	Advantages	Limitations
	Artificial	
General characteristics	Reduce habitat substrate variability influence Eliminate subjectivity in collection process Patchiness reduced Skill level required is less Long exposure periods (6–8 weeks) Discriminate between sediment and water toxicity	Habitats may be different, thus promotes growth of different species, not representative of site. Two trips needed Long exposure periods (6–8 wks) Sediment substrate effects, including toxicity, reduced Sampler loss through vandalism or sedimentation
Modified	Reduces compounding effects of substrate differences, multiplate sampler	Long exposure time, difficult to anchor, easily vandalized
Fullner	Wider variety of organisms	Same as modified Hester–Dendy
Basket Type	Comparable date, limited extra material for quick lab processing. Large amount of biomass.	No measure of pollution on strata, only community formed in sampling period, long exposure time, difficult to anchor, easily vandalized
Periphyton	Floats on surface, easily anchored, glass slides exposed just below surface	May be damaged by craft or flows, easily vandalized
	Natural	
Any bottom or sunken material	Indicate effects of pollution, gives indication of long-term pollution	May be difficult to quantitate; possible lack of growth, not knowing previous location or duration of exposure

Modified from EPA. *Handbook for Sampling and Sample Preservation of Water and Wastewater.* Environmental Monitoring and Support Lab, U.S. Environmental Protection Agency, Cincinnati, OH, EPA 600/4-82/029. 1982; EPA. *Ecological Assessment of Hazardous Waste Sites.* Environmental Research Laboratory, U.S. Environmental Protection Agency, Corvallis, OR. EPA 600/3-89/013. 1989a.

Figure 6.123 Stream net sampler.

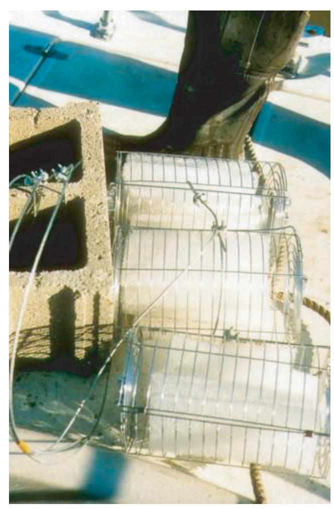

Figure 6.124 Artificial substrates (polyethylene mesh) in BBQ baskets secured to cinder blocks.

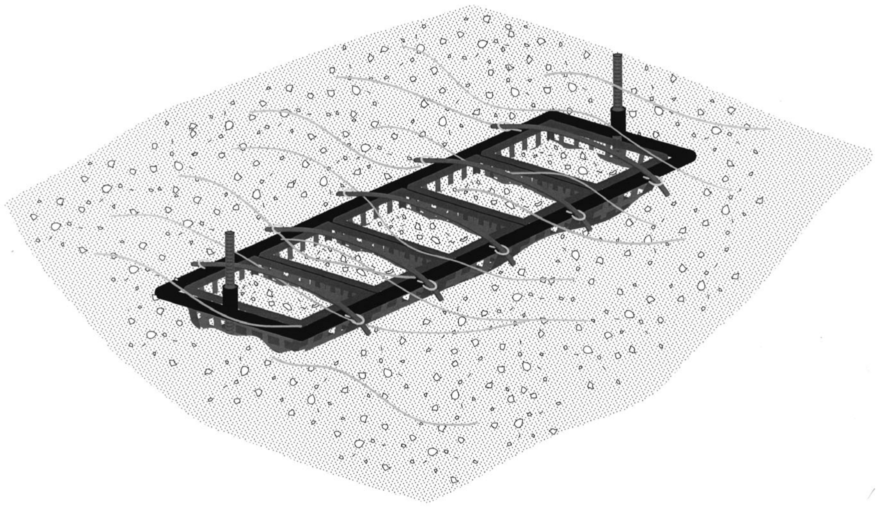

Figure 6.125 Colonization trays buried to stream sediment surface and secured with iron rods. Baskets are filled with cleaned substrates representative of the reference or test site.

Figure 6.126 Periphyton sampler, two styrofoam floats with eight glass microscope slides in rack.

Figure 6.127 Periphyton sampler in place, plus Hester–Dendy samplers.

Table 6.48 Comparison of Net Sampling Devices

Devices	Application	Advantages	Disadvantages
Wisconsin net	Zooplankton	Efficient shape concentrates samples	Qualitative
Clarke-Bumpus	Zooplankton	Quantitative	No point sampling, difficult to measure depth of sample accurately

From EPA. *Handbook for Sampling and Sample Preservation of Water and Wastewater.* Environmental Monitoring and Support Lab, U.S. Environmental Protection Agency, Cincinnati, OH, EPA 600/4-82/029. 1982.

ZOOPLANKTON SAMPLING

The zooplankton community plays a major role in the food web and aquatic ecosystem dynamics. Its use as an indicator of pollution in lotic systems has been limited. Studies are more common in lentic systems; however, they are complicated by a high degree of spatial and temporal variability, and less knowledge of pollution tolerances as compared to the benthos. The cladocerans, *Daphnia magna*, *Daphnia pulex,* and *Ceriodaphnia dubia*, have been useful as sensitive toxicity surrogate species. If an intensive lake–reservoir ecosystem effect study is to be conducted, they should be included. Commonly used sampling nets are listed in Table 6.48 and Figure 6.128.

Figure 6.128 Net sampler for plankton.

FISH SAMPLING

The fish community is perhaps the most important component of the ecosystem as viewed by public opinion, commercial interests, and regulatory requirements. In reality, however, it is no more important than any other major level of biological organization and is not as useful as other groups when evaluating stormwater effects. Fish, by nature, are in general a more transitory species than other aquatic organisms and, therefore, produce more variable results in biosurveys. Because they are mobile, they are often able to avoid polluted waters. This avoidance behavior makes evaluations of site-specific sources and problems more difficult. Sampling methods vary in their degree of efficiency and selectivity and compound data variance problems (EPA 1989c). They do, however, possess many advantages in the assessment process:

- Beneficial uses of stream segments characterized in terms of fisheries
- Many endangered species exist
- Effective collection methods exist
- Effective quality assessments are possible using community structure and functional metrics to form an index of integrity
- Used as regulatory and monitoring tools for decades; an extensive database exists on life history, distribution, and effects
- Indicators of long-term exposures and watershed conditions
- Comprise multiple trophic levels
- Drive ecosystem dynamics in the “top-down” approach theory and may integrate effects from lower trophic levels (“bottom-up” approach)
- Contaminant source to humans
- Useful for sublethal, chronic pollutant exposure effect studies

Many fish communities contain multiple trophic levels, such as invertivores, planktivores, herbivores, omnivores, and piscivores (Table 6.49; Karr et al. 1983). Trophic guild information is useful for evaluating system alterations at a functional and structural level. The omnivore component typically increases as water quality declines. Streams with fewer than 20% omnivores are often of good quality, and poor if greater than 45% are true omnivores (Karr 1981). There is also often a strong inverse correlation between the abundance of insectivorous cyprinids (minnows) and water quality (more abundant minnow populations indicate worse water quality). Another generality of feeding type and water quality is the presence/absence of top carnivores, which are at the top of the aquatic food chain and thereby integrate lower trophic level effects. They are most likely to show biomagnified toxicants in their tissue, but might not necessarily show effects from those toxicants. The validity of these generalizations has been well documented in the agricultural Midwest. However, there are exceptions nationwide. Tissue residues are good indicators of exposure for some nonpolar organics and methyl mercury; however, many metals and organics that can be metabolized cannot be assessed well with tissue information.

Sampling of fish communities is relatively labor intensive, often requiring special equipment and expertise. But, given the importance of fish in ecosystem structure and functioning, sport and commercial fishing, and public perceptions, they should be monitored.

Generally, the preferred sampling season is mid to late summer, when stream and river flows are moderate to low, and less variable than during other seasons (EPA 1990b). Although some fish species are capable of extensive migration, fish populations and individual fish may remain in the same area during summer (Funk 1957; Gerking 1959; Cairns and Kaesler 1971). However, large river, lake, and harbor habitats promote greater migration ranges. Ross et al. (1985) and Matthews (1986) found that stream fish assemblages were stable and persistent for 10 years, recovering rapidly from droughts and floods, indicating that large population fluctuations are unlikely to occur in response to purely natural environmental phenomena. However, comparison of data collected during different seasons is discouraged, as are data collected during or immediately after major flow changes (EPA 1989a).

Although various collection methods are routinely used to sample fish; electrofishing (Figures 6.129 through 6.131), seines (Figure 6.132), and rotenone (a poison) are the most commonly used methods in freshwater habitats (Tables 6.50 and 6.51). Each method has advantages and disadvantages (Nielsen and Johnson 1983; Hendricks et al. 1980). However, electrofishing is recommended for most fish field surveys because of its greater applicability and efficiency, and the good recoverability of stunned fish that are returned to the water (EPA 1989a,c).

Table 6.49 Trophic Guilds Used by Schlosser (1981, 1982a, 1982b) to Categorize Fish Species

Herbivore–Detritivores (HD)	HD species feed almost entirely on diatoms or detritus.
Omnivores (OMN)	OMN species consume plant and animal material. They differ from GI species in that, subjectively, greater than 25% of their diet is composed of plant or detritus material.
Generalized insectivores (GI)	GI species feed on a range of animal and plant material including terrestrial and aquatic insects, algae, and small fish. Subjectively, less than 25% of their diet is plant material.
Surface and water column insectivores (SWI)	WSI species feed on water column drift or terrestrial insects at the water surface.
Benthic insectivores (BI)	BI species feed predominantly on immature forms of benthic insects.
Insectivore–Piscivores (IP)	IP species feed on aquatic invertebrates and small fish. Their diets range from predominantly fish to predominantly invertebrates.

Figure 6.129 Electrofishing with backpack unit in main stream reach (notice nearby seine to capture stunned fish).

Figure 6.130 Electrofishing with backpack unit in near-shore areas.

Figure 6.131 Boat electrofishing unit. (Courtesy of Wisconsin Department of Natural Resources.)

Figure 6.132 Fish seining.

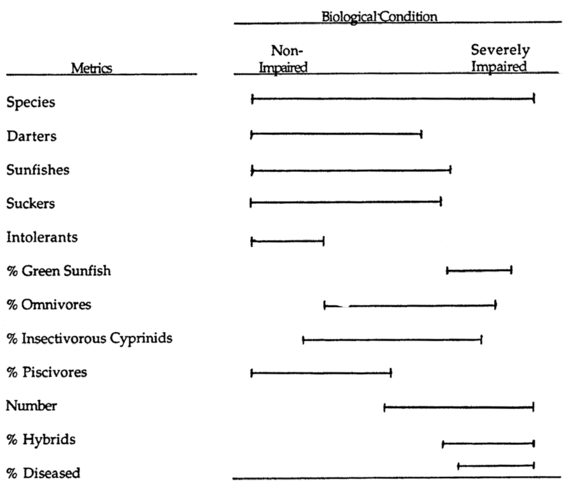

Figure 6.133 Range of sensitivities of Rapid Bioassessment Protocol V fish metrics in assessing biological condition. (Modified from EPA. *Ecological Assessment of Hazardous Waste Sites.* Environmental Research Laboratory. U.S. Environmental Protection Agency, Corvallis, OR. EPA 600/3-89/013. 1989a.)

Indices of Fish Populations

Perhaps the most popular index is the IBI. A slightly modified version is used in the EPA Rapid Bioassessment Protocols for fish. The IBI is weighted on the basis of individual species' tolerances to water and habitat quality. The IBI is comprised of 12 metrics, as follows:

A. Species richness and composition
   1. Species number
   2. Darter species number
   3. Sucker species number
   4. Sunfish species number
   5. Intolerant species number
   6. Green sunfish proportion
B. Abundance and condition
   1. Individual numbers
   2. Hybrid proportion
   3. Proportion with disease anomalies
C. Trophic composition
   1. Omnivore proportion
   2. Insectivorous cyprinid proportion
   3. Piscivore proportions

Table 6.50 Fish Sampling Methods

Methods	Advantages	Disadvantages
Electrofishing	Greater standardization of catch per unit of effort Less time and manpower than some sampling methods Less selective than seining (although it is selective toward size and species) Adverse effects on fish are minimized Appropriate in a variety of habitats	Sampling efficiency is affected by turbidity and conductivity. Initial cost of equipment Although less elective than seining, electrofishing is size and species selective. Effects of electrofishing increase with body size. Species specific behavioral and anatomical differences also determine vulnerability to electroshocking A hazardous operation that can injure field personnel if proper safety procedures are ignored
Reformed seining	Relatively inexpensive Lightweight and are easily transported and stored Repair and maintenance are minimal and can be accomplished on-site Restricted water quality parameters Effects on the fish population are minimal because fish are collected alive and are generally unharmed	Previous experience and skill, knowledge of fish habitats and behavior, and sampling effort are probably more important in seining than in the use of any other gear Sample effort and results are more variable than sampling with electrofishing or rotenoning Generally restricted to slower water with smooth bottoms, and is most effective in small streams or pools with little cover Standardization of unit of effort to ensure data comparability is difficult
Rotenoning	Effective use independent of habitat complexity Greater standardization of unit of effort than seining Provides more complete censusing of the fish population than seining or electrofishing	Kills all fish and possibly nontarget species, should only be used if other methods are not appropriate and if the data are essential Prohibited in many states Application and detoxification can be time and manpower intensive Effective use affected by temperature, light, dissolved oxygen, alkalinity, and turbidity High environmental impact; concentration miscalculations can produce substantial fish kills downstream of the study site

From EPA. *Ecological Assessment of Hazardous Waste Sites.* Environmental Research Laboratory, U.S. Environmental Protection Agency, Corvallis, OR. EPA 600/3-89/013. 1989a.

Table 6.51 Sampling Methods for Fish[a]

Method	Habitat	Persons	Time (hr)
Electrofishing	Small streams	2	0.25–1
	Large streams, rivers, lakes	2	0.25–1
Seining	Small streams or impoundments	2–3	0.50–1
Hoop net	Streams or rivers	2–3	2[b]
Gill, trammel nets	Lakes[d]	2–3	2–4[c]
Fyke net	Lakes[d]	2–3	2[c]

[a] Taken from Lagler (1978); Hendricks et al. (1980); Hubert (1983); Nielsen and Johnson (1985).
[b] Time for obtaining fish sample; time for stationary netting techniques includes time spent setting and receiving nets. It does not include time required to process sample (weighing, measuring, or taxonomic identification), which can range from 1 to 4 hours depending on taxonomic resolution and number of fish obtained.
[c] Time for hoop, gill, trammel, and fyke nets does not include 24 hours or period for which net is left in water to obtain sample.
[d] Gill, trammel, and fyke nets can also be used in some cases in flowing water if properly anchored; however, debris usually makes these applications troublesome.

From EPA. *Protocols for Short-Term Toxicity Screening of Hazardous Waste Sites.* Environmental Research Laboratory, U.S. Environmental Protection Agency, Corvallis, OR. EPA 600.3-88/029. 1989b.

Each metric is scored as 1 (worst), 3 (moderate), or 5 (best) as compared to the reference site or other data (see Fausch et al. 1984) showing regional norms (Table 6.52). Therefore, the index may range from 12 to 60 after all metric scores are totaled. Regional modifications have been developed by Hughes and Gammon (1987), Leonard and Orth (1986), Steedman (1988), and Wade and Stalaup (1987). The IBI is shown generally in Figure 6.134 and described in detail in Appendix C.

The Index of Well-Being (IWB), developed by Gammon (1976), was also developed in the midwestern United States to evaluate environmental stress effects on riverine fish. It is simpler than the IBI, using four measures: numbers of individuals, biomass, and the Shannon diversity index based on numbers and weight. Unfortunately, in some systems, high numbers and biomass of pollution-tolerant species produce a high index value, yet quality is reduced. To deal with this problem the Ohio EPA (1989) and Gammon (1989) developed a modified IWB which eliminates highly tolerant species, exotic species, or hybrids from the numbers and biomass components of the IWB, but retained in the Shannon index calculations. This modification has proven to be an effective assessment tool, which is consistent and sensitive to a wide range of environmental stresses. These equations are listed below:

Index of Well-Being:

$$\text{IWB} = 0.5 \ln N + 0.5 \ln B + H\,(\text{no.}) + H\,(\text{wt.})$$

where N = relative number of all species
B = relative weight of all species
H (no.) = Shannon index based on relative numbers
H (wt.) = Shannon index based on relative weight

Shannon Diversity Index:

$$\overline{H} = -\sum \left(\frac{n_i}{N}\right) \ln \left(\frac{n_i}{N}\right)$$

where n_i = relative numbers or weight of the *i*th species
N = total number or weight of the sample

The IBI and mIWB require that indigenous fish species be classified in terms of environmental tolerance (to both natural and anthropogenic stressors). Tolerance levels (Appendix C) vary with each species, between ecoregions, seasonally, at different life stages, and they depend on the presence of other stressors, organism health, and the type of stressor. This group of critical variables makes any “tolerance” classification crude and tenuous. Nonetheless, the use of these classifications has been effective in evaluating ecosystem impairment. For many systems, shifts in dominant species and trophic classification away from sensitive, nonomnivores (e.g., trout, walleye) to tolerant omnivores (e.g., carps), clearly and easily show impairment exists. In other areas, where impairment is just beginning, as in a stream reach downstream of acute effects (“gray” zone), and where ecosystem recovery is beginning, the species tolerance levels will be uncertain.

TOXICITY AND BIOACCUMULATION

Why Evaluate Toxicity?

Toxicity and bioaccumulation evaluations are important and often essential components of stormwater impact assessments. They produce information that cannot be accurately determined or extrapolated from other assessment components. Toxicity tests have strengths and weaknesses that must be recognized (Table 6.53). If there is a clear understanding of the test responses and associated assumptions, and if proper QA/QC is followed, toxicity testing will allow for sensitive, meaningful, and efficient assessments of ecosystem quality and will identify stressor magnitude frequency, and duration. The science of aquatic toxicology has progressed rapidly in recent years and is now an integral component of many EPA regulatory programs. Toxicity testing may evaluate effects and address a wide variety of study objectives, using any of several general and specific monitoring approaches (Table 6.54). This variety of approaches allows for a high number of different component combinations, with each possibly providing unique information and having different assumptions associated with them. Many different approaches and organisms have been used for toxicity testing, and these will be discussed in the following section. Figures 6.135 through 6.138 show several test setups used in the Environmental Health Sciences laboratories at Wright State University, while Figures 6.139 and 6.140 are two of the Azur Environmental procedures, using phosphorescent phytoplankton, used in the environmental engineering labs at the University of Alabama at Birmingham.

Odum (1992) stated that stress is usually first detected in sensitive species at the population level. Natural population and community responses are not measured directly with whole effluent toxicity (WET) tests (La Point et al. 1996, 2000). The traditional surrogates (*P. promelas* and *C. dubia*) may not be as sensitive as indigenous species (Cherry et al. 1991). Indirect effects of toxicity on species, population, and community interactions can be important (Clements et al. 1989; Clements and Kiffney, 1996; Day et al. 1995; Fairchild et al. 1992; Giesey et al. 1979; Gonzalez and Frost 1994; Hulbert 1975; La Point et al. 2000; Schindler 1987; Wipfli and Merritt 1994), and may not be detected by WET testing. A huge ecological database exists showing the importance of species interactions in structuring communities (e.g., Dayton 1971; Power et al. 1988; Pratt et al. 1981).

It is less likely that strong relationships will exist between WET test responses and indigenous communities at sites where there are other pollutant sources, where effluent toxicity is low to moderate, or where dilution is high. Based on fish and benthic invertebrate responses, several studies suggest that WET tests are not always predictive of receiving water impacts (Clements and Kiffney 1994; Cook et al. 1999; Dickson et al. 1992, 1996; Niederlehner et al. 1985; Ohio EPA 1987); however, many studies have shown WET tests to be predictive of aquatic impacts (e.g., Birge et al. 1989; Diamond et al. 1997; Dickson et al. 1992, 1996; Eagleson et al. 1990; Schimmel and Thursby 1996; Waller et al. 1996). These differences should not be surprising however, as it is likely a result of WET test organisms and field populations experiencing different exposures (Burton et al. 2000; EPA 1991e). In an effluent-dominated system, the in-stream exposure is very similar to a WET test. A less degraded watershed, or one that is not dominated by point sources, may have sensitive indigenous populations that are exposed to "toxic" effluents at nontoxic concentrations. Conversely, if sensitive species have already been lost from a watershed, a toxic effluent may be inhibiting their return. In highly degraded sites, virtually any traditional assessment tool (acute toxicity testing, chemical concentrations, indigenous communities) will show effects and strong correlations with other tools. The WET tests were not developed to evaluate all natural and anthropogenic stressors nor to show all biological responses (such as mutagenicity, carcinogenicity, teratogenicity, endocrine disruption, or other important subcellular responses). In addition, highly nonpolar compounds may elicit an effect in short-term exposures. These issues dictate that additional assessment tools be utilized in order to protect aquatic ecosystems (Waller et al. 1996).

Table 6.52 Regional Variations of IBI Metrics

Variations in IBI Metrics	Midwest	New England	Ontario	Central Appalachia	Colorado Front Range	Western Oregon	Sacramento San Joaquin
1. Total Number of Species	X	X		X	X		X
# native fish species			X			X	
# salmonid age classes						X	X
2. Number of Darter Species							
# sculpin species						X	
# benthic insectivore species		X					
# darter and sculpin species			X				
# salmonid yearlings (individuals)						X	X
% round-bodied suckers	X						
# sculpins (individuals)							X
3. Number of Sunfish Species	X				X		
# cyprinid species						X	
# water column species		X					
# sunfish and trout species			X				
# salmonid species							X
# headwater species	X						
4. Number of Sucker Species	X	X				X	
# adult trout species						X	X
# minnow species	X				X		
# sucker and catfish species			X				
5. Number of Intolerant Species	X	X			X	X	
# sensitive species	X						
# amphibian species							X
Presence of brook trout			X				X
6. % Green Sunfish							
% common carp						X	
% white sucker		X			X		
% tolerant species	X						
% creek chub				X			
% dace species			X				

7. % Omnivores	X	X	X	X	X	X	
% yearling salmonids					X	X	
8. % insectivorous Cyprinids	X						
% insectivores		X				X	
% specialized insectivores				X	X		
# juvenile trout							X
% insectivorous species	X						
9. % Top Carnivores	X	X	X				
% catchable salmonids						X	
% catchable wild trout							X
% pioneering species	X						
Density catchable wild trout							X
10. Number of Individuals	X		X	X	X	X	X
Density of individuals		X					
11. % Hybrids	X	X					
% introduced species					X	X	
# simple lithophils	X						
% simple lithophilic species	X						
% native species							X
% native wild individuals							X
12. % Diseased Individuals	X	X	X	X	X	X	
13. Total Fish Biomass						X	

Note: X = metric used in region. Many of these variations are applicable elsewhere.

From EPA. *Rapid Bioassessment Protocols for Use in Streams and Rivers: Benthic Macroinvertebrates and Fish*. Office of Water, U.S. Environmental Protection Agency, Washington, D.C. EPA 444/4-89/001. 1989c.

Select a Site

Identify Regional Fish Fauna

Assign Species to Trophic, Tolerance, and Origin Guilds

Assess Available Data for Metric Suitability and Stream Size Patterns

Develop Scoring Criteria from Reference Sites

Quantitatively Sample Fish

List Abundances of Species, Hybrids, and Anomalies

Calculate and Score Metric Values

METRIC SCORES (IBI)

Metric	Scoring Criteria[a] 5	3	1
1. Number of native fish species	>67%	33-67%	<33%
2. Number of darter or benthic species	>67%	33-67%	<33%
3. Number of sunfish or pool species	>67%	33-67%	<33%
4. Number of sucker or long-lived species	>67%	33-67%	<33%
5. Number of intolerant species	>67%	33-67%	<33%
6. Proportion of green sunfish or tolerant individuals	<10%	10-25%	>25%
7. Proportion omnivorous individuals	<20%	20-45%	>45%
8. Proportion insectivores	>45%	20-45%	<20%
9. Proportion top carnivores	>5%	1-5%	<1%
10. Total number of individuals	>67%	33-67%	<33%
11. Proportion hybrids or exotics	0%	0-1%	>1%
12. Proportion with disease/anomalies	<1%	1-5%	>5%

[a] Metrics 1-5 are scored relative to the maximum species richness line. Metric 10 is drawn from reference site data.

INDEX SCORE INTERPRETATION[a]

IBI	Integrity Class	Characteristics
58-60	Excellent	Comparable to pristine conditions, exceptional assemblage of species
48-52	Good	Decreased species richness, intolerant species in particular; sensitive species present
40-44	Fair	Intolerant and sensitive species absent; skewed trophic structure
28-34	Poor	Top carnivores and many expected species absent or rare; omnivores and tolerant species dominant
12-22	Very Poor	Few species and individuals present; tolerant species dominant; diseased fish frequent

[a] From Karr et al. 1986; Ohio EPA 1987.

Recommendations

Figure 6.134 Flowchart of bioassessment approach advocated for Rapid Bioassessment Protocol V. (From EPA. *Rapid Bioassessment Protocols for Use in Streams and Rivers: Benthic Macroinvertebrates and Fish.* Office of Water, U.S. Environmental Protection Agency, Washington, D.C. EPA 444/4-89/001. 1989c.)

Table 6.53 Strengths and Weaknesses of Toxicity Tests in Stormwater Assessments

Strengths	Weaknesses
Toxicity can be quantified and linked to the presence of specific or multiple contaminants, sources, or affected media (i.e., soil, water, sediment, vegetation, aquatic biota); an important assessment component needed to establish causality.	Measure of potential toxic effects on resident biota at the test site; however, cannot always be directly translated into an expected magnitude of effects on populations in the field.
Response is an integrated index of bioavailable contamination, whereas chemical analyses measure only total concentrations of specific compounds.	Results are dependent on specific techniques, e.g., test species, collection method, water or sediment quality, test duration, etc.
More sensitive than biosurvey methods.	If surrogate species used, there is a question of their response relationship to indigenous species.
Sensitive in all types of aquatic ecosystems.	Single species test responses may not relate to community structure and ecosystem function impacts.
Results are specific to the location at which the sample was collected; thus they can be used to develop maps of the extent and distribution of bioavailable contamination and toxic conditions.	May not detect long-term toxicity, bioaccumulation, sublethal effects, or persistent, hydrophobic contaminants.
Temporal toxicity dynamics of stormwater events can be quantified and correlated with flow and other physicochemical characteristics.	Laboratory exposure conditions in toxicity tests are not directly comparable to field exposures; additional confounding variables and other stresses are important in the field.
Indigenous species may be tested in the laboratory or *in situ.*	
Approach effectively used by the EPA and many states to regulate point source pollution.	
Multiple species, multiple trophic levels, and multiple levels of biological organization (e.g., plant, bacteria to fish) may be evaluated.	
Results are easily interpreted and amenable to QA/QC; within- and among-laboratory precision estimates are already available for several tests.	
May be tested *in situ,* thus reducing laboratory-sample handling related artifacts.	
Acute toxicity tests are relatively quick, easy, and inexpensive to conduct; results from acute tests are used as a guide in the design of chronic toxicity tests.	
Chronic and short-term chronic toxicity tests are generally more sensitive than are acute tests, and can be used to define "no effect" levels; in addition, chronic tests provide a better index of field population responses and more closely mimic actual exposures in the field.	
In situ and laboratory exposures may be used to assess bioaccumulation.	
May reveal recent short-term toxicity events that are not detected in biosurveys.	
Have a long regulatory use in the NPDES program	

Table 6.54 Problem Definition: Toxicity Test Approaches

Assessment Component	Monitoring Approach
Test media	Effluent (e.g., point source discharges of wastewater or runoff)
	Ambient water
	Sediment
	Interstitial water
	Extractable fraction (e.g., elutriate)
	Soil
	Sludge
	Sample fractionation (e.g., the EPA's Toxicity Identification Evaluation procedures)
Test organism	Surrogate
	Indigenous to ecoregion
	Resident
	Single species
	Multiples of single species
	Communities or populations
	Multitrophic and/or multiple levels of biological organization
Effect level	Acute (lethality endpoint)
	Short-term chronic (e.g., growth or reproduction during partial life cycle
	Chronic (sublethal endpoint during full life cycle)
	Biomarker (sublethal endpoint in short-term exposure)
	Concentration response defined (e.g., LC50, NOEL[a]) vs. exposure to undiluted (100%) sample
Test environment	Laboratory:
	Static, static-renewal, recirculating, or flow-through
	Water only
	Water (reconstituted or site water)[b] and sediment (suspended[c] or bedded[d])
	In situ:
	Effluent mixing zone
	Ambient water only
	Sediment only
	Water and sediment
	Artificial substrate
Measured endpoints	Functional
	Population-community structure
	Organism
	Cellular or molecular

[a] Sample concentration with 50% lethality, no observable effect level.
[b] Allows separation of water and sediment toxicity.
[c] Suspended solids concentration physically maintained or fluctuates.
[d] Mixed, sieved, or intact core.

Figure 6.135 Fathead minnow rearing tanks at Environmental Health Sciences laboratories at Wright State University.

Figure 6.136 Adult fathead minnow rearing tank at Wright State.